Development of Hydroxyapatite Nanorods–Polycaprolactone Composites and Scaffolds Derived from a Novel In-Situ Sol-Gel Process

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Abstract: Hydroxyapatite (HA) is the most substantial mineral constituent of a bone which displays splendid bio-compatibility and bioactivity properties. Nevertheless, its mechanical property is not utmost appropriate for a bone substitution. Therefore, a composite consist of HA and a biodegradable polymer is usually prepared to generate an apt bone scaffold. In the present work polycaprolactone (PCL) was employed as a matrix and hydroxyapatite nanorods were used as a reinforcement element of the composite. HA/PCL nanocomposites were synthesized by a new in-situ sol-gel process using low cost chemicals. Chemical and physical characteristics of the nanocomposite were studied by X-ray diffraction (XRD), field emission scanning electron microscopy (FE-SEM) and Fourier transform infrared (FTIR) analyses. XRD analysis revealed that pure hydroxyapatite with no undesirable compound was formed within the nanocomposite. Moreover, hydroxyapatite had low crystallinity with the average crystallite size of 62.5 nm. FE-SEM images showed dispersion of HA nanorods in PCL matrix with suitable interaction were obtained. The average length and diameter of HA nanorods were calculated 167 nm and 53 nm, respectively. It was found that HA/PCL nanocomposite had macroporous structure and high surface area which are essential parameters for cell attachment and protein absorption. Biological properties of HA/PCL scaffolds, prepared through a solvent casting process, were investigated under in vitro condition. Bioactivity of these scaffolds was studied in a saturated simulated body fluid (5×SBF). It was confirmed that HA/PCL scaffold was uniformly covered with a layer of calcium phosphate crystals with the thickness of few microns and phase composition of hydroxyapatite. Consequently, scaffolds met the requirements of materials for bone tissue engineering and could be used for many clinical applications in orthopedic and maxillofacial surgery.

Key words: polymer-matrix composites, nanocomposites, sol-gel methods.

1. Introduction

Tissue engineering offers a new approach to regenerate diseased or damaged tissues such as bone. The rapidly growing research in the bone tissue engineering area thus provides a new and promising approach for bone repair and regeneration. Bone is a natural organic–inorganic ceramic composite consisting of collagen fibrils containing embedded, well-arrayed, nanocrystalline, rod-like inorganic materials 25-50 nm in length. Hydroxyapatite (HA) is chemically similar to the inorganic component of bone matrix, a very complex tissue with general formula Ca_{10}(OH)_{2}(PO_4)_6. HA particles on nanometric scale (nHA) have been proved to be an osteoconductive material that also chemically binds to enamel and dentine. The biologically beneficial characteristics of nHA, such as being the major inorganic component of the bone matrix, its specific affinity toward many adhesive proteins, and its direct involvement in the bone cell differentiation and mineralization processes, make nHA especially attractive for applications in the bone regeneration field. 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. However, they have not adequate biomechanical properties (i.e., high brittleness, low fatigue strength, and low flexibility) and do not encounter the mechanical requirements for direct-loading applications, as well as application of dynamic force during the in vitro bone tissue engineering process. Recent advances in nanoscience have reignited interest in the formation of nanosized HA and the study of its properties on the nanoscale.

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received considerable attention. For instance, in column chromatography application, rod shaped HA shows enhanced protein absorption due to their charging surface efficiency. HA crystals with nanorod features have shown desirable biocompatibility and bioactivity because of higher absorbability, since the underlying van der Waal’s interactions are proportional to surface area of the rods. Furthermore, HA in human tooth and bone is found in the form of nano-polycrystalline hexagonal nanorods. Polycaprolactone (PCL) is a biodegradable polymer with remarkable toughness and good biocompatibility. It is a semi-crystalline aliphatic polymer with sustained biodegradability, good biocompatibility and expectant mechanical strength that has a slower degradation rate and higher fracture energy than most biocompatible polymers.

Sol-gel process is one the appropriate methods for synthesis of biomaterials as the final product shows chemical uniformity. Moreover, high level of interaction makes it possible to carry out the procedure at the low temperature. The sol-gel derived HA compounds usually show small grain size in the range of submicrons or even nanometric which would be adopted to the host tissue more rapidly. Meanwhile, it has been reported that, dispersion of particles has impressive effects on mechanical and bioactivity properties of scaffolds. The ideal porous scaffolds for hard tissue engineering should be biocompatible, biodegradable and absorbable. In addition, a suitable microstructure of scaffold (including their porosity, pore size and interconnection between pores), the sufficient mechanical strength retaining for a period and good cell-scaffold interaction are also the necessary for bone tissue engineering application. 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 hard tissue regeneration materials. Many bone tissue engineering scaffolds have been made as composites, either by the introduction of nHA or HA within polymeric matrices or by the mineralization of HA nanoparticles on the surface of polymeric substrates. For example, Fabbi et al. synthesized PCL/HA composites by in situ generation of HA in the polymer solution starting from the precursors calcium nitrate tetrahydrate and ammonium dihydrogen phosphate and the solvent tetrahydrofuran (THF) via sol–gel process. XRD pattern of PCL/HA composite revealed a strong calcium deficiency in the inorganic phase generated in situ inside the polymer solution, being brushite (CaHPO₄·2H₂O). Raucci et al. reported HA/PCL hybrid composite material using calcium nitrate tetrahydrate and di-phosphorous pentoxide as precursors and ethanol as the solvent. They observed a trace of calcium carbonate in the hybrid composite. Wang et al. prepared porous nHA/PCL scaffolds with different composition ratios of nHA/PCL via an ex-situ method (i.e., melt-molding/porogen leaching technique). PCL with molecular weight 50000 and nano-hydroxyapatite with particle size 40-60 nm (Nanjing Emperor Nano Material Co. Ltd., China) were used as starting materials and poly-ethylene glycol (PEG20000) was used as the fugitive agent. PCL has an intrinsic hydrophobic chemical nature, and its poor surface wetting and interaction with biological fluids avoid cells adhesion and proliferation. For this reason, and in order to get enhanced mechanical properties, PCL is often used as polymer matrix in composites including osteogenic and osteoinductive inorganic phases, such as HA to confer its high bioactivity to the polymer-based composite promoting bone regeneration. Therefore, biomechanical characteristics of scaffold are higher than those of pure HA. Some chemical and physical properties of nHA/PCL scaffolds are evaluated in previous studies; accordingly, it is pointed out that by increasing nHA content of composite the degrading rate of composite will be increased due to facilitating water to infiltrate into the scaffolds. After employing HA/PCL scaffolds as part of a tissue, they are able to maintain their suitable mechanical properties for a proper time. Once HA particles are dispersed in the polymer matrix; poor interfacial adhesion is often observed as a consequence of the different chemical nature of the components and their different surface energy, resulting in a too fast decay of the mechanical properties of the composite. For this reason, an in situ synthesis of HA by sol–gel process directly in the presence of the polymer solution, appears to be a promising strategy for the achievement of homogeneous hybrid materials. This procedure should avoid the extensive particles agglomeration typically seen in HA/PCL composites obtained by mechanical incorporation of preformed HA powders into the polymer melt or solution, causing non homogeneous materials. Moreover, in a sol-gel process, particle size is controlled directly by the means of the interaction between calcium and phosphate precursors under controlled temperature and pH conditions.

So far, HA/PCL hybrid composite containing HA nanorods has not been reported by an in-situ sol-gel process. Here, a new strategy for synthesis of HA/PCL composite, containing HA nanorods, is introduced by sol-gel method using non-alkoxide precursors (i.e., calcium hydroxide and phosphoric acid) and acetone as the solvent. One of the advantages of this method is the use of an alternative to alkoxide precursors as calcium and phosphorous sources. Besides controlling the phase structure, composition homogeneity, monodispersity and microstructure, the cost of the product is an important concern in developing technologies for synthesis of HA/PCL nanocomposites. There-

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