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Micro- and nano-injectable composite biomaterials containing calcium phosphate coated with poly(DL-lactide-co-glycolide)

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Abstract

Calcium phosphate/poly(DL-lactide-co-glycolide) (CP/DLPLG) composite biomaterial, in which each CP particle was coated with DLPLG, was synthesized. Two kinds of composites were prepared: microcomposite, with particles 150–200 μm in size, and nanocomposite, with the particles 40 ± 5 nm in size. Using nanoparticles, a new class of injectible composite biomaterials was produced. Based on scanning electron microscopy, atomic force microscopy, differential thermal analysis, thermogravimetric analysis, differential scanning calorimetry and Fourier transform infrared analyses, the structure and phase organization in both biomaterials was identified and in both studied cases CP particles were coated with DLPLG polymer. An injectable composite biomaterial, the characteristics of which depend on the ratio of the phases, was prepared by mixing physiological solution with the nano-CP/DLPLG composite. Rheological studies indicated a possible agglomeration of particles of the injectable nano-CP/DLPLG composite biomaterial with a CP content of 65%.

Keywords: Bone; Calcium phosphate; Composite; Copolymer; Nanocomposite

1. Introduction

The use of calcium phosphates (CP) in medical treatments is currently an interesting field of research aimed at developing different biomaterials for the reconstruction of human tissue. Human bone tissue is a prime example of a nanocomposite system in the human body. Calcium phosphate crystals, typically a few nanometers in width, incorporated into the polymer matrix are the key components of human bone tissue [1]. The use of calcium phosphate in bone regeneration dates back to 1920 [2]. Good osteoconductive characteristics of this biomaterial have enabled the development of a wide range of CP-based composite biomaterials [3]. Biphasic calcium phosphate (BCP), composed of hydroxyapatite (HAp) and tricalcium phosphate (TCP), can be a successful alternative to allografting due to its good osteoconductive characteristics [4].

Products of the degradation of bioresorbable polymers based on lactide acid (PLLA or DLPLG) in the presence of CP can increase the acidity of the environment, but, due to their nature, can be easily removed metabolically and are not toxic [5,6]. Calcium phosphate mixed with DLPLG polymer intensifies the activity of alkaline phosphatase more than CP alone, which is important for the differentiation of osteoblasts that dictates the regeneration process within the organism [7]. CP/DLPLG composite biomaterials based on biphasic CP (BCP) or hydroxyapatite as a ceramic CP component exhibit good adhesion to human cells, indicating the high level of biocompatibility of these materials [8,9]. CP powder mixed with bioresorbable polymer (DLPLG or similar) fibers in the form of cement with macropores provides suitable conditions for vascular tissue growth [10]. DLPLG mixed with CP can be used as a carrier in tissue engineering [11].

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CP/DLPLG composites have been used as bone filler, in which each BCP granule is of the order of a micrometer in size and was coated with DLPLG polymer. They proved successful in in vitro and in vivo tests for repair of bone defects [12,13]. CP/DLPLG also has a lesser cytotoxicity than pure CP [14].

Micro/nanoparticles of CP or CP-based composite biomaterials mixed with a liquid phase such as physiological solution, blood plasma or growth factor could be a usable form of injectable biomaterial with controlled hydrodynamic properties. Injection of this type of material could improve the process of reconstruction of some bone defects. By the addition of the growth factor to the composite biomaterial, good osteoconductive characteristics of CP could become osteoinductive ones [15]. The response of the organism depends on the interaction of the biomaterial with the surrounding tissue and its adhesion to cells. Besides composition and morphology, the size of the composite particles plays a key role in theses phenomena. Nanoparticles have several advantages over microparticles in interactions between the biomaterial and the organism. New methods of chemotherapy and cancer treatment are based on the use of spherical DLPLG nanoparticles [16]. Nanoparticles have unusual properties that can be exploited to improve drug delivery. Because of their size, they are often taken up by cells where larger particles would be excluded or cleared from the body. Small molecules, proteins and nucleic acids can be loaded into nanoparticles that are not recognized by the immune system [17].

Until now, injectable composite biomaterials based on CP and bioresorbable/non-bioresorbable polymers have been the subject of investigations aimed at obtaining injectable biomaterials with satisfactory mechanical and biological characteristics [18,19]. Of special interest is their application in the substitution of bone tissue both in dentistry and medicine due to the rapid formation of new bone tissue [20]. Injectable biomaterials based on BCP or bioresorbable polymer are of special interest in bone tissue engineering. Their advanced characteristics come from their good mechanical properties and biocompatibility and the ease of tissue regeneration [21]. Good osteoconductive characteristics of injectable biomaterials based on microparticles of BCP and poly(ε-caprolactone) have been confirmed in preliminary in vivo investigations. In these investigations the rheological properties as a function of the weight fraction of the solid phase in the biomaterial were analyzed and the potential use of this biomaterial in bone tissue reconstruction was demonstrated [22]. As a primary strategy aimed at improving the characteristics of injectable composite based on CP, Bohner and Baroud [23] suggested a decrease in particle size. Recently, more attention has been paid to CP systems mixed with DLPLG used as fillers, which have become useful in the treatment of a wide range of bone defects [24].

In our earlier studies the possibility of synthesizing CP/DLPLG composite biomaterial in the form of granules 150–200 μm in diameter was shown [25] and indications were given regarding how to prepare the same material but in the form of particles up to 100 nm in size. Spherical nanoparticles could be suitable for obtaining injectable biomaterials. The aim of this paper is to investigate the possibility of synthesis of a composite biomaterial in which each particle of CP is coated with bioresorbable DLPLG polymer. The possibility of obtaining composites of the same composition but of different particle sizes (micro- and nanoparticles) was studied. The microstructure, qualitative composition and thermal characteristics of the obtained particles were analyzed. CP nanospheres were coated with a thin polymer layer by a modified emulsion procedure to obtain spheres smaller than 50 nm in size. Using these nanospheres, injectable biomaterials of different solid/liquid phase ratios were prepared which could be used in the reconstruction of bone defects. Such materials represent a new class of injectable nanocomposite materials. The rheological characteristics of these injectable composite biomaterials have also been studied.

2. Materials and methods

A CP gel was produced by precipitation of calcium nitrate and ammonium phosphate in an alkaline medium at 100 °C [26,27]. DLPLG (50:50) (Sigma Chemical Company, USA) was used as a polymer component. Polyvinyl alcohol (PVA) with a 98% degree of hydrolization was also used.

Micro- and nanocomposites were obtained according to procedures 1 and 2.

2.1. Procedure 1

The gel was dried, granulated at room temperature and calcined at 1150 °C for 6 h. Granules of CP were added into the completely dissolved polymer to 80 mass% [25]. The suspension was mixed at a velocity of 30 rev min^{-1}, and then methanol was added. Afterwards, PVA (0.02%) was added to the suspension (DLPLG/PVA = 10/1). After the solvent evaporation, the granules were dried at room temperature for 24 h. Granules ranging from 130 to 180 μm in size were used in further investigations. The granules of CP/DLPLG composite biomaterial, sizes from 150 to 250 μm were separated by sieves.

2.2. Procedure 2

The CP gel was added into the completely dissolved polymer to 80 mass%. The suspension was mixed at a velocity of 1200 rev min^{-1}, and then methanol was added. Afterwards, PVA (0.02%) was added to the suspension (DLPLG/PVA = 10/1). The addition of methanol into the three-component system of solvent–polymer–CP caused its thermodynamic destabilization. This induced sedimentation of the polymer onto the CP particles, thereby covering them. After the solvent evaporation, the particles were dried at room temperature for 24 h.

ID	Title	Pages
2476	Micro- and nano-injectable composite biomaterials containing calcium phosphate coated with poly(dl-lactide-co-glycolide)	9