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Production, by co-grinding in a media mill, of porous biodegradable polylactic acid–apatite composite materials for bone tissue engineering

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ABSTRACT

This paper presents the results of a study of the production of porous biodegradable composite materials by co-grinding, followed by scaffolding. Dry powders of polylactic acid and nanocrystalline carbonated apatite, analogous to bone mineral were co-ground in a tumbling ball mill in order to disperse the mineral filler within the polymer. Porous scaffolds were then made by hot moulding the mixture of the two components along with a pore-forming agent which was subsequently eliminated by washing. The mechanical resistance of the scaffolds was evaluated in order to determine the best operating conditions to produce implants offering optimised properties for use as bone substitutes. It was shown that 30 wt.% of filler and 70 wt.% of pore-forming agent produce scaffolds which are sufficiently porous and resistant.

1. Introduction

Polymer nanocomposites were first developed in the late 1980s and the recent scientific discoveries and technical breakthroughs have allowed simple commodity plastics to be turned into 'hi-tech' materials with much improved properties.

The improvement of the biological activity and performance of bone substitute materials is one of the main concerns in orthopaedic and dental surgery specialists. Among a large variety of biomaterials, an increasing number of bioceramics are specifically prepared to give a biological activity promoting the integration of the implants into biological tissues and favouring their repair. The association of calcium phosphates (CaP) with organic molecules (polymer or protein), as mineral-organic composites, can impart bioactive properties and mechanical properties (i.e. better structural integrity and flexibility) closer to those of bone as compared to pure calcium phosphate ceramics.

Recently, such composites have gained increasing interest in the field of tissue engineering which appears as a promising concept for bone reconstruction. This concept requires a biodegradable host matrix (composite for example) acting temporarily as a mechanical support and directing osteoblast cell growth and tissue neoformation once implanted. High porosity is necessary to promote the osteointegration of the implant and especially neoformed bone invasion. Furthermore the shape, porous architecture and pore morphology are important factors for the performance of tissue engineering supports. Numerous studies related to porous scaffolds based on composites CaP-protein or polymer (e.g. HAP-collagen, -cellulose, -polyethylene, -polysaccharides, -polyesters) can be found in the literature [1–3].

Generally, composite materials (polymer-ceramic) are obtained by melt blending or dispersion of the strengthening agent in the polymer in solution. Especially in the case of composites for bone tissue engineering, other techniques inspired by in vivo hard tissue calcification processes have emerged. For example, organised organic matrices with numerous sites favourable for nucleation of calcium phosphates after phosphatation or silanation treatments were used [4]. Many studies can be found in the literature where the aim is to produce biomimetic artificial bone-like tissue involving HAP and collagen as fiber, gel or gelatin (denatured collagen) [5–7].

Macroporosity can be created within the composite using different methods: solvent casting/particulate leaching, emulsion freeze drying or thermally induced phase separation.

Biodegradable porous composites including resorbable polymer such as polylactic acid (PLA) and/or polyglycolic acid (PGA) and a resorbable apatite can be prepared at ambient temperature [8,9]. This kind of association has the advantage of a controlled pH in the surrounding medium during the composite degradation, since apatite (whose degradation involves the release of alkaline components) can moderate the pH drop resulting from polyester (PLA or PGA) biodegradation (hydrolysis of ester bond). Other good reasons for using such synthetic or natural polymers are: a) the ease of processing, b) the possibility of controlling the polymer degradation rate depending on its composition (polymer or copolymer), molecular weight and

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crystallinity. Moreover, in vivo degradation of PLA releases lactic acid, which is a natural metabolite that the organism can eliminate by natural ways. The biomaterial 3D architecture (dense or porous composite) also has an effect on the rate of biodegradation which is lower in the case of porous composites where the acid released from PLA degradation can be leached with biological fluids whereas this acid concentrated in the bulk of dense composite should autocatalyse composite biodegradation. However to date such composites have found only limited industrial development.

The formation of a polymer-apatite composite can also correspond to a first step in the preparation of nanocrystalline apatite porous ceramic. For example, Tadic et al. [10] reported the preparation of nanocrystalline apatite-based porous bioceramics using both sodium chloride salt and polyvinyl alcohol fibers as water-soluble pore-forming agents and cold isostatic pressing without the need to sinter.

Properties of composites are strongly dependent, not only of the interfaces between the matrix and the filler (i.e. affinity between the two components), but also of the size distribution of fillers. A way that has not yet been explored is to prepare such composite biomaterials by the use of co-grinding polymer and mineral filler. This process has been used for polymer matrix composites including calcite [11,12]. It is based on the principle of alternated fragmentation and agglomeration of particles in a mill. The materials are introduced into a mill as powders, and are first fragmented by the grinding medium. However, the materials involved do not have the same hardness, leading to different grinding rates. The fragmentation of a mineral is faster than that of a polymer. Thus, the mineral fragments adhere to the polymer particles and undergo in their turn a fragmentation. Consequently, the as-produced mineral fines agglomerate on the free faces of the polymer particles, thus slowing down the agglomeration of the latter and supporting even their fragmentation. Then, these successive stages of fragmentation and agglomeration of the mineral continue, generate a progressive covering of the polymer particles by mineral particles. A coating phenomenon thus operates. If the operation is continued, agglomeration can occur between the coated polymer particles. Each agglomerate thus obtained is a composite in which the filler, of very small size, is well distributed in the matrix. It has been shown [12] that co-grinding greatly improves the composite mechanical properties compared to that obtained by a simple mixing process, since the dispersion of the filler in the matrix and its adhesion on the polymeric surface are better. Moreover, the limit minimum size that can be reached by the filler fragments in the case of co-grinding is divided by 10 compared to the size that can be obtained when grinding the mineral alone, improving the homogeneity of the composite.

Nanocrystalline apatites are the major inorganic constituent of mineralised tissues in vertebrates. Synthetic biomimetic nanocrystalline apatites exhibit enhanced and tunable reactivity as well as original surface properties related to their composition and mode of formation. These apatites offer extensive possibilities for the design of biomaterials with improved bioactivity using unconventional processing at low temperature, preserving their surface reactivity and biological properties and enabling their association with active molecules and/or ions [13,14]. All these interesting properties justified the choice of nanocrystalline carbonated apatite analogous to bone mineral as filler for the production of the composite presented in this study.

In the present study we investigated in the possibility of producing porous biodegradable composites for bone tissue engineering by co-grinding in a tumbling ball mill and shaping at low temperature. This leads to several specific characteristics: control of porosity and of pore distribution, good mechanical resistance. The choice of PLA-nanocrystalline apatite association presents several advantages: polymer matrix biodegradability, biological and mechanical properties of the mineral filler. Sodium chloride particles were used in this study as pore-forming agent, as it is easily dissolved by leaching.

2. Experimental procedure

The polymeric matrix used in this study is poly-L,D-lactic acid (PLA, Galactic) with a molecular weight of 90,000 g/mol, initially available in the form of spherical beads having a diameter of about 3 mm. The beads were pre-ground in a laboratory blade mill (Janke and Kunkel). A pre-grinding temperature of 18 °C was fixed by water circulating in a double jacket surrounding the mill chamber to avoid heating and even melting of the particles in the contact with the blades. Pre-grinding was performed for 10 min and the particles were then sieved. Only those having a size less than 250 µm were selected for this study. The initial mean size of the preground product is 157 µm. A scanning electron microscopy (SEM) micrograph of preground particles is shown in Fig. 1.

The homopolymer of L-lactide (LPLA) is a semicrystalline polymer: Poly-L-lactide is about 37% crystalline, with a melting point of 173–178 °C and a glass-transition temperature of 60–65 °C. PLA based materials exhibit high tensile strength and low elongation and consequently have a high modulus making them more suitable for load-bearing applications such as in orthopaedic fixation and sutures [15]. DL-PLA is an amorphous polymer exhibiting a random distribution of both isomeric forms of lactic acid, and is unable to arrange into an organised crystalline structure. This material has lower tensile strength, higher elongation, and a much more rapid degradation time, making it more attractive for drug delivery applications: the degradation time of LPLA is requiring more than 2 years to be completely absorbed.

The mineral charge is nanocrystalline carbonated apatite which has been prepared at ambient temperature and physiological pH by double decomposition of a diammonium hydrogenphosphate and sodium hydrogencarbonate (90 g of $(NH_4)_2HPO_4$ and 90 g of $NaHCO_3$ in 1500 mL of deionised water) and a calcium nitrate solution (52.2 g of $Ca(NO_3)_2\ 4H_2O$ in 750 mL of deionised water). The calcium solution is rapidly poured into the phosphate and carbonate solution at room temperature (20 °C) and stirred only for a few minutes. The apatite was left for 15 days to mature after precipitation at room temperature in the mother solution without stirring and in a stoppered vial to minimise the release and uptake of CO_2 at physiological pH. At the end of maturation period, the precipitate is then filtered under vacuum and washed with deionised water (2 L). Then the gel is freeze-dried and finally stored in a freezer to prevent further maturation of the apatite before its use.

The carbonate content of the as-prepared apatite (4.5% w/w of CO_3), was determined using a CO_2 coulometer (UIC Inc., USA). Calcium concentration was determined by complexometry with EDTA and the phosphorus concentration by visible spectrophotometry of the phospho-vanado-molybdenum complex. The Ca/P atomic ratio of

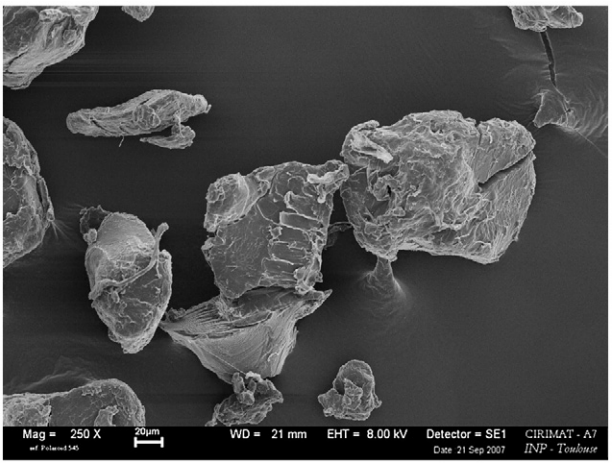

Fig. 1. SEM micrograph of preground polymer particles.