Crystallization of bioinspired citrate-functionalized carbonate-apatite nanoparticles with tailored carbonate content

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Abstract

Novel citrate-functionalized carbonate-apatite nanoparticles with mean lengths ranging from 20 to 100 nm were synthesized by a thermal-decomplexing batch method. Needle-like and plate-shaped morphologies were obtained in the absence and presence of sodium carbonate in the precipitation medium, respectively. The precipitation time and the presence of sodium carbonate strongly affect the chemical composition as well as the dimensions and the crystallinity of nanoparticles. At a short precipitation time, poorly crystalline apatites of 100 nm mean length with a low degree of carbonation (1.5% w/w, mainly in B-position) and a high citrate content (5.9% w/w) were precipitated. This citrate content is close to that recently measured in bone apatite. When increasing the precipitation time up to 96 h the mean length and the citrate content progressively decrease and at the same time the nanoparticles become more crystalline. They are composed of a well-ordered carbonate-substituted apatitic core embedded in a non-apatitic hydrated layer containing citrate ions. This layer progressively transforms into a more stable apatite domain upon maturation in aqueous media. The nanoparticles displayed excellent compatibility properties in cell biological systems, since they were not cytotoxic to a mouse carcinoma cell line when added to a final concentration of 100 µg ml⁻¹. This work provides new insights into the role of citrate on the crystallization of nanobone apatites. Moreover, the synthesized nanoparticles are promising materials for use as nanocarriers for local targeted drug delivery systems as well as building blocks for the preparation of nanostructured scaffolds for cells in bone tissue engineering.

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1. Introduction

Hydroxyapatite (HA, Ca₅(PO₄)₃(OH)) is the most stable calcium phosphate phase in physiological conditions and the model compound used to denote the mineral component of bone and dentin [1,2]. Biological apatites are calcium-deficient crystals with a Ca/P ratio lower than the theoretical value of stoichiometric hydroxyapatite (1.67) [1,2]. They are plate-shaped nanoparticles, with a length of ~30–50 nm, a thickness of ~30–50 nm and a thickness of 2–10 nm [2,3]. In addition, they usually introduce several foreign ions into the crystalline structure, such as carbonate (4–6%), Na (0.9%) and Mg (0.5%) [2,3].

Apatite crystallization has been the subject of extensive research in numerous interdisciplinary areas to better understand its formation mechanism in natural mineralization processes [4], as well as to investigate its preparation as a biomaterial due to its well-known biomedical properties [5]. Many different methodologies have been proposed to prepare nanosized and/or nanocrystalline apatites, both in the absence and in the presence of additives [6]. However, the synthesis of apatite presenting characteristics similar to those of biological bone in terms of sizes, specific surface area, carbonation degree and surface composition is still a technological challenge.

It is well accepted that the organic component of bone acts as an important regulator of lattice orientation, particle size, and size distribution in biomineralization processes [7,8]. However, the details of molecular recognition at organic–inorganic interfaces are still not completely understood. In the last few years, most researchers have focused on the effects of amino acids [9] and biochemical macromolecules such as carboxylate-rich proteins [10–12] on calcium phosphate nucleation and growth, but have disregarded the role of small organic molecules such as citrate. Citrate ions are Ca-complexing agents that are biocompatible, but inhibit apatite crystal growth by strongly interacting with the surface [13,14]. Recently, new insight about the role of citrate has