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On the use of nanoliposomes as soft templates for controlled nucleation and growth of hydroxyapatite nanocrystals under hydrothermal conditions

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Abstract

Calcium phosphates are biocompatible materials with the composition closest in similarity to the mineral phase of bone. Among them, hydroxyapatite (HA) is shown to be the most promising bioactive compound widely used in bone tissue engineering applications. It has been shown that the preparation of HA with controlled morphology and size distribution may be beneficial to therapy of bone disease. In this study, a new method was developed for the synthesis of nano-HA particles in which nanoliposomes were used as nucleation sites for growth of HA crystals. Phase composition, morphology, particle size and the molecular structure of sediments were studied using X-ray diffraction techniques (XRD), scanning electron microscopy (SEM), transmission electron microscopy (TEM) and Fourier transform infrared spectroscopy (FTIR). The results showed that nanoliposomes could act as carriers for the crystal growth of nano-HA particles. The powder produced from liposome encapsulation contained hexagonal bipyramidal structures of HA with nanometer dimensions, spherical shapes, dense morphologies and a mean size of about 60 nm. Further investigation of the bioactivity and biocompatibility of this new class of biomaterials is being performed.

Keywords: Hydrothermal method; Hydroxyapatite; Nanoliposomes; Nanostructure

1. Introduction

Hydroxyapatite (HA) is known to be the most important member of calcium phosphates' family. HA is the main constituent of natural bone composition and is similar to natural bone tissue [1,2]. It is highly biocompatible and is widely used in bone implants. Recent advancements in nanoscience and nanotechnology have reignited investigations of nano-scale HA formation in order to clearly define the small-scale properties of HA. It has been suggested that nano-HA may be an ideal biomaterial due to its good biocompatibility and bone integration ability [3,4]. Nano-HA particles have attracted the attention of many scientists due to their increased molecular purity and mechanical properties of implants [5–8]. Size and shape of HA crystals are critical factors that impact the physical–chemical properties of the final product such as fracture strength, fracture toughness, surface characteristics, biocompatibility, and solubility of the particles.

HA powders have various morphologies such as needle-like, spherical, plate-like, etc. [9]. Nano-HA particles are synthesized through several methods such as wet chemistry or hydrothermal methods. In these methods, in order to improve mechanical properties, rod-shaped HA was synthesized with Cetyltrimethylammonium bromide (CTAB), used as a controlling factor for the nucleation and growth of HA crystals [10–12]. A micelle is an aggregate of surfactant molecules dispersed in a liquid colloid. A surfactant, CTAB creates micellar structures which would act as nano-reactors for the synthesis of nanoscale HA [13]. When the CTAB concentration is close to the critical micelle concentration (CMC), the particles are almost spherical in shape. This could be explained in view of the fact that, at CMC, CTAB in aqueous solutions forms spherical micelles and, therefore, the obtained

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particles are essentially spherical with particle sizes ranging from 69 nm to 102 nm [14]. At concentrations above the CMC, spherical micelles turn into rod micelles.

The nano-rods form aggregates with a low surface area. Wang et al. [15] and Sun et al. [16] reported synthesis of nano-HA powders using cationic surfactants. In both cases, however, the particle shapes could not be controlled. Ye et al. produced well-defined HA nano-rods (20–50 nm in diameter and 200–500 nm in length) [17]. In recent years, investigators have used patterns or micelles to control the shape and size of nano-HA particles. To do this a surfactant has been used as a pattern maker to control the nucleation and growth of HA crystals. When the surfactant molecules enter an aqueous solution, the cationic heads form a hard layer where the phosphate ions construct a layer around this hard shell, due to the interaction of non-equivalent charges that serves as the center of nucleation. When a solution containing calcium ions is added, HA particles are formed around the micelles [18].

The precipitates synthesized both with and without liposomes are poorly crystalline, and have a similar chemical composition to the natural HA. Lipid vesicles composed of bilayers of phospholipid molecules, named 'liposomes', have good biocompatibility due to the lipids being one of the major components of biological membranes [19]. In order to improve the nucleation process for new bone formation, Huang et al. [19] utilized liposome-coated HA and tricalcium phosphate as bone implants carried out in the mandibular bony defect of miniature swine, concluding biocompatibility of liposome-coated materials with clinical endpoint enhancements in comparison with those in the absence of liposomes. Preparation of HA coated with liposomes with controlled morphology and size distribution may be beneficial to therapy of bone disease and anticancer applications [20]. One of the main characteristics of powders synthesized via hydrothermal methods is their high degree of crystallinity, making them suitable for the preparation of biomaterials that are under shear stress. However, the disadvantage of conventional hydrothermal methods is the use of high-temperatures for synthesizing HA powders with desirable properties. This defect poses problems in industrializing the hydrothermal method, since equipments that can withstand such temperatures and pressures are highly expensive. On the other hand, Chu and Liu [20] presented a low degree of crystallinity of HA, produced as one of the disadvantages of synthesizing HA, using liposomes, at room temperature.

Hydration stage has a higher impact on the types of composed liposome layers, their sizes and their volumes. Dried lipid nature, surface area and porosity are also important. The speed of the water phase addition, temperature, and ionic conditions have an impact on the rate of formation and the morphology of the resulting HA particles [19–22]. Since a molecular dipole stands as the nucleation point for HA crystal growth, we studied liposomes as points of nucleation in order to create new ways to grow these crystals. A mixture of lipid deposits in a volatile organic solvent on the surface of a flask in a vacuum rotary evaporator under low pressure. Multi-layered liposomes with sizes ranging from tens of microns to a few tenths of microns are shaped with the addition of a large volume of water to the dried lipids formed inside the flask.

In this study, by combining the hydrothermal method and using the nanoliposome process, a novel industrial and economical method is proposed. In summary, in this method, preparation of liposome was done through dehydration of a dry film and hydration of a dried lipid film via an aqueous solution of calcium. Then, an aqueous solution of sodium phosphate was added dropwise into the liposome solution so that HA crystals germinated and grew inside the liposome chamber. The obtained solution was kept at 120 °C resulting in nano-HA particles with high crystallinity.

2. Materials and methods

2.1. Synthesis process

Round-bottom flask and beakers, washed in distilled water, were placed in an oven at 121 °C to prevent any contamination which could affect the size of the particles produced. The containers were left to cool for 15 min. To make the dried lipid film,Dipalmitoylphosphatidylcholine (DPPC) (lipoid) and Cholesterol (Merck) were dissolved in chloroform. In order for the solvent to evaporate and dried lipid film to be formed, the resulting solution was poured into a round-bottom flask and put on a hotplate stirrer for 30 min at a temperature of 41 °C. For the preparation of hydroxyapatite, calcium nitrate tetrahydrate ($Ca(NO_3)_2 \cdot 4H_2O$) (Merck Prolabo 22 384.298) and Diammonium hydrogen phosphate ($(NH_4)_2HPO_4$) (Merck Prolabo 21 306.293) were used as sources of calcium ions and phosphate ions, respectively. An aqueous solution of calcium salt was prepared using deionized water and added to the dried lipid film and mixed at a temperature of 51 °C for 2 h by a stirrer. The pH of the solution was adjusted to 4.5 using pure acetic acid. The obtained solution was sonicated for 30 min (placed under ultrasound waves). Liposomal nanoparticles, due to their high surface to volume ratio, have a very high tendency to stick together. Ultrasound liposomal causes nanoparticles to spread in liquid media and form nano-scale structures. Aqueous solution of sodium phosphate was prepared as deionized water drops were added to a solution of calcium and lipids. The milky suspension without aging was poured into the autoclave chamber made of stainless steel with Teflon lining. It was then placed inside the drying chamber for 22 h at a temperature of 120 °C. At the end of the hydrothermal process, the suspension was precipitated using a centrifuge and the obtained precipitate was washed three times with ethanol and then three times with deionized water and finally, dried in a dryer at a temperature of 90 °C, for 22 h. The precipitate was hand grounded and prepared for the experiments.

2.2. Characterization

The chemical composition and the degree of crystallization of the resulting powder were measured using the X-ray diffraction (XRD) method with CuKα radiation (0.15406 nm

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