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Preparation, characterization and antibacterial properties of silver nanoparticles–hydroxyapatite composites by a simple and eco-friendly method

Flávio Augusto Cavadas Andrade[a,*], Luci Cristina de Oliveira Vercik[b], Fernando Jorge Monteiro[c,d], Eliana Cristina da Silva Rigo[a,b]

[a]*Interunidades Bioengenharia, EESC/FMRP/IQSC – Universidade de São Paulo, Av. Trabalhador São Carlense 400, 13566-590 São Carlos, SP, Brazil*
[b]*Departamento de Ciências Básicas, FZEA – Universidade de São Paulo, Av. Dq. Caxias Norte 225, 13635-900 Pirassununga, SP, Brazil*
[c]*Departamento de Engenharia Metalúrgica e Materiais, FEUP – Faculdade de Engenharia, Universidade do Porto, Rua Dr. Roberto Frias s/n, 4200-465 Porto, Portugal*
[d]*INEB – Instituto de Engenharia Biomédica, Rua do Campo Alegre 823, 4150-180 Porto, Portugal*

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Abstract

Hydroxyapatite (HA) has been widely used as an implant material due to its high chemical similarity with the mineral component of bone extracellular matrix. Silver nanoparticles (AgNPs), when used in adequate amounts, are known to provide beneficial effects on biomaterials due to their powerful antimicrobial activity. In this work, HA powder was obtained by chemical precipitation and AgNPs were synthesized in colloidal suspension in the presence of chitosan. The HA powder was immersed in as-prepared AgNPs colloids to produce silver nanoparticle–hydroxyapatite composites (HA-AgNPs) with theoretical silver proportion ranging from 0.016 to 0.40 wt%. The AgNPs synthesis and adsorption onto HA were studied by a UV–vis technique, while the presence of silver in HA-AgNPs was confirmed by ICP-AES and EDS analysis. XRD and TEM characterization showed no significant changes in the structure and morphology of HA after AgNPs incorporated. High-resolution SEM showed spherical AgNPs (5–10 nm diameter) and TEM analysis revealed nanosized rod-shaped HA particles (100–150 nm long and 40–50 nm wide). A good antibacterial ability was observed in all materials against two bacterial strains (*Escherichia coli* and *Staphylococcus aureus*) using the disk diffusion method. Considering the simple methodology involved in the production of these materials, it is suggested that the HA-AgNPs can be used for development of antibacterial biomaterial applications.

Keywords: Nano-hydroxyapatite; Silver nanoparticle; Antibacterial effect; Adsorption

1. Introduction

Inorganic biomaterials based on calcium orthophosphate have their wide range of applications in medicine and bone tissue engineering [1]. Among them, synthetic hydroxyapatite (HA, $Ca_{10}(PO_4)_6(OH)_2$) is the most promising due to its composition and chemical structure similar to the mineral phase of bone [2–4]. This bioactive ceramic has long been used for repairing and reconstructing bone fractures as well as for coating orthopedic, maxillofacial and dental implants [2,5–7]. However, one of the biggest current problems in the biomedical field is post-surgical infections arising from recent-implanted synthetic biomaterials, since these provide sites for potential bacterial adhesion [8]. Such infections often lead to severe pain, bone tissue loss and eventually require implants removal, consequently raising morbidity. To overcome the limitations of antibiotics used in prevention and treatment of these infections that might lead to highly resistant bacteria [9], special attention is dedicated in controlling the release of alternative antimicrobial agents, such as copper, zinc and silver, all presenting wide activity and low bacterial resistance [10,11]. Several studies reported the intentional inclusion of chemical

*Corresponding author. Tel.: +55 16 98823 5617; fax: +55 19 3565 4117.
E-mail address: flavio.andrade@usp.br (F.A.C. Andrade).

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elements into biomaterials in order to obtain antimicrobial effects [12–14], and silver has been the most common due to its strong and nonselective antibacterial activity [15]. Furthermore, the reactivity of silver is even more efficient when used in nanometer-sized particles due to their high surface-to-volume ratio that allows better contact with microorganisms [16]. Besides, silver nanoparticles (AgNPs) are less toxic at low concentrations [17,18]. An interesting and preferable synthesis route for obtaining AgNPs should use more environment friendly reagents, for instance the natural biopolymer chitosan [19–21]. Various forms of silver phosphates have already been used, from precipitation and co-precipitation in aqueous solution [22–25] to ionic exchange [26,27], by thermal coating [28] and cold spraying [29], besides precipitation of HA with silver nanoparticles [13,30–32]. However, the use of adsorption techniques involving the immersion of HA in solutions of Ag and AgNPs to produce antibacterial biomaterials is yet very little explored [27,33,34]. Simple processes involving the immersion of powder in colloids of AgNPs became attractive since they do not require the use of sophisticated technological equipment, and use low cost chemical reagents. Concerning the importance of antibacterial biomaterials for biomedical application, the main goal in this work was to develop a simple method for the synthesis of silver nanoparticles–hydroxyapatite composites with antibacterial properties. During this procedure four powders of HA-AgNPs were obtained by introducing low amounts of AgNPs through an adsorption process. These materials were evaluated regarding their physicochemical characteristics as well as the in vitro antibacterial activity, by using Gram-negative (*E. coli*) and Gram-positive (*S. aureus*) bacterial strains.

2. Materials and methods

2.1. Hydroxyapatite synthesis

Hydroxyapatite was synthesized through a wet chemical precipitation method using slow addition of phosphoric acid (H_3PO_4) into a calcium hydroxide solution ($Ca(OH)_2$) and Ca/P 1.67. All of the chemicals used were of analytical grade. After dripping, the solution was kept under constant stirring at room temperature for 6 h (aging). The precipitate was removed by filtration (Whatman Filter Paper, 14 μm pore size), washed twice with distilled water to remove the excess of ions and contaminants and dried at 100 °C for 24 h. The solid obtained was deagglomerated in an agate mortar, sieved (80-mesh) and heat-treated at 800 °C during 3 h with a heating rate of 15 °C/min.

2.2. Synthesis of colloidal silver nanoparticles

Chitosan (QS) (Practical grade > 85% deacetylated, Polymar S/A) was prepared in a concentration of 6.92 mg/mL in acetic acid 1% v/v (Synth), and stirred-up until complete solubilization. Silver nitrate solution (Synth) was prepared at 52 mmol/L in distillated water.

Silver nanoparticles were synthesized according the process of Wei and Qian [20], with some modifications. Briefly, the solution of silver nitrate was mixed for 30 min at room temperature with QS (volume ratio of 2:5), leading to a concentration of 14.86 mmol/L. The mixture was transferred to glass tubes and it was kept at rest at 90 °C for different times (1–24 h) in a temperature controlled bath. After synthesis, the AgNPs colloids were stored at room temperature and in dark glass tubes.

2.3. Preparation of silver nanoparticles embedding in hydroxyapatite

The AgNPs obtained with 6 h of synthesis were chosen to prepare the samples of silver nanoparticles–hydroxyapatite composites (HA-AgNPs). Four dilutions were performed using distilled water and the 6 h-colloid (Table 1). Ten grams of HA powder were immersed in each dilution at room temperature and kept under constant stirring for 30 min. These samples were named HA-AgNP01, HA-AgNP05, HA-AgNP10 and HA-AgNP25 in accordance with the addition of 1, 5, 10 or 25 mL of AgNPs colloid, respectively.

The HA-AgNPs suspensions were filtered in a 14 μm filter paper (Wathman®) and washed extensively with deionized water to remove non-adsorbed AgNPs. The filtrate was dried at 60 °C for 24 h and it was heat-treated (3 h at 800 °C, at 15 °C/min). Aliquots were collected before, during and after immersing the HA powder in AgNPs. For the antibacterial tests, 100 mg of HA-AgNPs powder was uniaxially pressed to obtain disks of 8.0 mm diameter using a semi-automatic hydraulic press (70 MPa). Afterwards, all disc samples were sterilized in an autoclave (120 °C, 20 min).

2.4. Sample characterization

UV–vis spectroscopy was performed using a DU 800 (Beckman Colter) spectroscope to confirm the synthesis of AgNPs through their surface plasmonic resonance (SPR) band and to detect the presence of AgNPs in the HA matrix. Crystallinity and phase identification of the powder materials (HA and HA-AgNPs) were performed by X-ray diffraction (XRD) using a Siemens D5005 diffractometer (Bruker AXS), with $Cu_{\alpha 1}$ ($\lambda = 1.5406$ Å) radiation. Data were collected for 2θ in the range of 20–60° with steps of 0.033° and step time of 1 s.

Freshly synthesized nanoparticles (6 h, 12 h and 24 h) and the aforementioned powder with the highest AgNP concentration (HA-AgNP25) were studied using a Magellan™ 400L XHR-SEM microscope (FEI Company). The size distribution and polydispersity of synthesized AgNPs was performed using the image processing program ImageJ 1.45s (NIH). Energy Dispersive Spectroscopy analysis was carried out using a

Table 1
Preparation of HA-AgNPs composites with the 6 h-AgNPs colloid synthesized.

Sample	AgNPs colloid (mL)	Water (mL)	$[Ag^+]$ (mmol/L)	m_{Ag} (mg)
HA-AgNP01	1	49	0.29	1.6
HA-AgNP05	5	45	1.48	8.0
HA-AgNP10	10	40	2.97	16.0
HA-AgNP25	25	25	7.43	40.0