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Silver ion impregnated composite biomaterial optimally prepared using zeta potential measurements

N. Sakthivel [a], R. Socrates [b], G.M. Shanthini [a], A. Rajaram [b], S. Narayana Kalkura [a,*]

[a] Crystal Growth Centre, Anna University, Chennai, 600 025, India
[b] Biophysics Laboratory, Central Leather Research Institute, Chennai 600 025, India

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ABSTRACT

Biodegradable, antimicrobial composite of various silver ion concentrations was synthesized using zeta potential and isoelectric point measurements, for a controlled release of silver ions, and in addition to assess the effect of protein adsorption with the increase of the silver ion concentration. The interaction between hydroxyapatite (HAp) and silver incorporated hydroxyapatite (AgHAp) with gelatin was increased by optimally adjusting the zeta potential and isoelectric point of the ceramic (HAp and AgHAp), and bio-polymer individually. The electrostatic interactions between the ceramic and biopolymer were confirmed, through shifts in N–H stretching, decrease in the swelling ratio, and increase in the degradation temperature observed by the derivative thermo-gravimetric analysis (DTG). These results substantiate that, the zeta potential is a novel tool to increase the ceramic–biopolymer interaction. Increasing electrostatic interaction between the biopolymer and ceramic, decreases the release of silver ions in the simulated body fluid, due to the controlled degradation of the biopolymer. The isoelectric point decreases with the increase of the silver ion concentration, which evidenced the change in the net surface charge. With the increase of the silver ion concentration, the protein adsorption decreases due to an increase in hydrophilic character of the composite. This study examines the minimum concentration of silver ion essential for maximum protein adsorption, antimicrobial and hemocompatibility. This study provides a novel route to control the release of silver ions by enhancing the ceramic–polymer interaction and estimate the silver ion concentration suitable for protein adsorption. The prepared composite is nontoxic, degradable, and antimicrobial, with the controlled release of silver ions in the simulated body fluid.

1. Introduction

Polymeric scaffolds can act as templates for cell material interaction and provide structural support for newly formed tissues [1]. Various research efforts focus on the preparation of scaffolds having maximum cell material interaction, high cell adhesion, minimum inflammation, high surface to volume ratio, less toxicity, and porosity between ~100–200 μm to enhance tissue ingrowth as in bone regeneration [2].

Composites of hydroxyapatite (HAp) and gelatin are widely used as implant materials due to their biocompatibility, adhesiveness, and hemostatic property. Hydroxyapatite is one of the most stable calcium phosphate salts, having bioactive, and osteoinductive properties, found in hard tissues like bone and teeth [3]. Gelatin is a biopolymer, obtained by the partial hydrolysis of structural fibrous protein like collagen, found to be in the skin, tendons, cartilages, bones and connective tissues [4]. The gelatin used in the present study is widely used as a space filler, and as a scaffold with ceramic, for tissue engineering [5].

The post-surgical infections detected in patients undergoing open surgical procedure, are around 5%. Wound infection can be prevented by the slow release of antibiotics by coating the implant with an antibiotic, or loading antibacterial beads which help the sustained slow release of antibiotics. The controlled release of antibiotics is achieved by synthetic biocompatible, non-biodegradable polymers as for example PMMA in fracture fixation [6]. The non-biodegradable polymers may not resorb as in the case of PMMA; hence, the removal of these polymers after healing may be essential [7]. Further, to solve the problem of infection, implants can be directly loaded with antimicrobial agents such as antibiotics. The adsorbed antibiotics are washed out by the body fluids; hence, post-surgical infections cannot be controlled in a long term. To avoid such problems, the proposed composite materials can be incorporated with antimicrobial metal elements such as Ag^{+}, Cu^{2+}, and Zn^{2+} [8]. Among these elements, ionic silver has been shown to control the growth of microorganisms very effectively, by strongly binding with the bacterial surface which leads to massive proton damage and complete de-energization [9]. The exposure of silver ions at higher concentration has been reported to cause irritation and local corrosion, but does not lead to argyria, which could be due to occupational exposure. A recent study reported that incorporation of silver ions in trace quantities in an implant material, may not cause harmful effects to humans

* Corresponding author.
E-mail addresses: kalkura@yahoo.com, kalkurasn@annauniv.edu (S. Narayana Kalkura).

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[10]. To the best of our knowledge, most of the AgHAp synthesized so far contained very high concentration of silver ions (above the toxic level): moreover, they did not possess the capacity to release silver ions in a sustained manner for long term (>7 days) [11–15].

In the present study, the novel preparation of silver ion incorporated gelatin–AgHAp composite by the zeta potential method was introduced for sustained release of silver ion. The effect of silver ion concentration on protein adsorption, hemocompatibility and swelling was investigated. The gelatin AgHAp composite devoid of antibiotics in the pore size ranging from 150–200 μm, which could be utilized for mineralization and osteoid tissue formation is proposed.

2. Experimental section

2.1. Preparation of hydroxyapatite ($Ca_{10}(PO_4)_6(OH)_2$)

Analar grade calcium nitrate ($Ca(NO_3)_2 \cdot 4H_2O$, Merck), phosphoric acid (H_3PO_4, Merck) silver nitrate (Merck), and gelatin (Merck) were used for the preparation of gelatin–HAp composite, without any further purification.

1 M calcium nitrate solution, and 0.6 M phosphoric acid with various concentrations of silver nitrate (0, 0.03, 0.06 and 0.12 M) were prepared. The calcium nitrate solution was added drop wise into the 0.6 M phosphoric acid solution having the various concentrations of silver nitrate, in 1:1 volume ratio and then pH was adjusted to 11 by using an ammonia solution. After adding calcium nitrate to the 0.6 M phosphoric acid solution, the mixture was stirred vigorously for about 3 h, and allowed to settle for 24 h. Similarly, AgHAp was prepared using 0, 0.03, 0.06, and 0.12 M silver nitrate, and denoted as 0AgHAp, 3AgHAp, 6AgHAp, and 12AgHAp respectively.

2.2. Preparation of gelatin–AgHAp composite

HAp slurry was prepared along with a gelatin solution, in the 1:1 weight ratio, and the pH was adjusted to 5.5. The resultant paste was poured into a Petri dish, as shown in Fig. 1. The mixture was allowed to gel at room temperature, subsequently frozen, and freeze dried. The freeze dried composites were cross linked with 0.3% glutaraldehyde, and stored in dark containers. Similarly, composites were prepared using gelatin and 0AgHAp, 3AgHAp, 6AgHAp, and 12AgHAp, denoted as G0SHAp, G3SHAp, G6SHAp, and G12SHAp.

2.3. Characterization

The XRD patterns of the HAp, AgHAp, gelatin–HAp, and gelatin–AgHAp were recorded using CuKα radiation (λ = 0.154 nm), 2 range of 15–70° with a scan speed of 2°/min, and a step rate of 0.02 per second, using the PANalytical X'pert powder XRD system.

The functional groups present in the silver doped hydroxyapatite and undoped hydroxyapatite and composites, were analyzed by JASCO FT/IR 6300 using the ATR technique in the frequency range of 4000–400 cm^{-1}.

The zeta potentials of HAp and AgHAp, were found by dispersing the powder in water at various pH. Similarly, gelatin solutions were prepared at various pH and their zeta potentials were recorded by the Malvern zetasizer nano ZS system. The obtained zeta potentials were plotted against the pH, and the pH at which the zeta potential was zero, indicated the isoelectric point.

The morphology of the scaffolds was observed, using the CARL ZEISS scanning electron microscope (SEM). The scaffolds were coated with gold using an ion sputter coater.

The thermo-gravimetric analysis (TGA/DTG) was performed between 40–800 °C in air at a heating rate of 20 °C/min using SDT Q600 (TA Instruments, WATERS).

2.4. Swelling

The water binding capacity of the composite was determined by measuring the swelling ratio. The pre-weighed scaffolds were incubated in PBS (pH 7.4). The incubated samples were taken out and the weight of the scaffold was noted after removing the excess water on the surface. The weight was continuously noted in a regular time interval until it reached an equilibrium value. The swelling ratio was calculated as follows [16].

$$\text{Swelling Ratio} = W_t - W_0 / W_0$$

W_t — weight of the scaffold after 't' time and W_0 — initial weight of the scaffold.

2.5. Hemolysis test

The hemolytic assay was performed, by using acid citrate dextrose human blood (ACD). The ACD solution was prepared by mixing 0.544 g of anhydrous citric acid, 1.65 g of trisodium citrate dehydrate, and 1.84 g of dextrose monohydrate. The ACD blood was prepared by adding 1 mL of ACD solution to 9 mL of fresh human blood. The scaffolds were equilibrated in normal saline. The saline was removed from the equilibrated samples, and then 0.25 mL of ACD blood was added and incubated for 20 min. Hemolysis was stopped by adding 2 mL of sterile saline to the incubated sample, which was allowed to stand for 1 h, and then centrifuged at 750 *g* for 5 min, and the scaffolds were recovered.

Human blood with deionized water served as the positive control, and the sterile saline served as the negative control. The supernatants were collected and the absorbance was measured using the UV spectrophotometer (UV-1601, Shimadzu) at 545 nm [17].

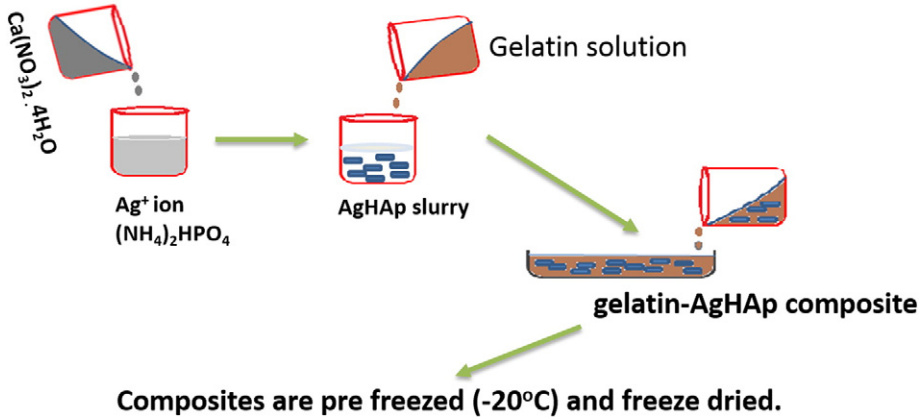

Fig. 1. Schematic representation of the synthesis process.