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Synthesis, phase stability of hydroxyapatite–silver composite with antimicrobial activity and cytocompatability

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Abstract

In the present study, the pH dependence hydroxyapatite (HAp) ceramics synthesis, and, their phase stabilities with respect to calcination temperatures were examined. Subsequently, HAp ceramics mixed with different silver amounts (HAp–Ag composites) were prepared in the temperature range 800–1200 °C to evaluate the phase stability of HAp in presence of silver. XRD results showed that as-synthesized HAp powder to be partially crystalline and are independent of pH of solution in the range of 9–11. These HAp phases remained stable even upto 1200 °C although crystallinity increased in all cases. Additional Ag peaks were appeared in HAp–Ag composites when the mixed powders were heated in the temperature range 800–1200 °C. FTIR result further confirmed the presence of Ca^{2+} and PO_4^{3-} ions. TEM observation showed the precipitation of needle like HAp nanocrystals of 20–40 nm in length which agglomerated into particulate morphology above 800 °C. Antibacterial study of HAp–Ag composites on *Staphylococcus aureus* for 1, 3 and 5 wt% silver concentration showed that 3 wt% silver is sufficient to kill the surrounding bacteria. Further studies on NIH3T3 cells for HAp–Ag composites at 10 wt% silver of different concentrations showed good cytocompatibility compared to HAp indicates the prospective applications in therapeutic interventions.

Keywords: Hydroxyapatite; Chemical precipitation; Antimicrobial study; Silver; TEM

1. Introduction

Hydroxyapatite [$Ca_{10}(PO_4)_6(OH)_2$, HAp] is a well-known calcium phosphate ceramics found as major component of bone and tooth [1,2]. When a bone fractured or cavity formed in the tooth, synthesized HAp powder is used as substitute material. This HAp powder alleviates the pain through the growth of bone-like apatite layer on its surface and gets integrated with the surrounding bone [3,4]. It has also been reported that porous HAp samples when implanted in bone defects show both osteoconductivity and osteoinductivity and directly bonds to living bone [5–7]. In some cases, composite material such as synthesized HAp ceramics mixed with polymers or metals are used for better mechanical integration as well as bioactivity when implanted in the body and provide an excellent platform for the existing bone to grow [8–12].

HAp ceramics being low in mechanical strength is very often used as coating on load bearing orthopedic implants to enhance the osteointegration in physiological condition [13–18]. This coated metal implant helps bone integration through the HAp layer, and, mechanical strength is provided by the base metal.

These bioactive HAp ceramics are prepared by several synthetic routes which include hydrolysis, hydrothermal synthesis, hydrothermal exchange, solid processes from bovine bone, sol–gel processes, and spray pyrolysis from the natural coralline and wet chemical methods [19–25]. Among them, wet chemical precipitation of powders from the salt solutions is the simplest method for rapid synthesis of large amounts of HAp powder in a controlled manner at room temperature [26].

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However, bacterial infections during the synthesis of HAp ceramics or at any stage of implant preparation or during the implantation by the surgeons are sometimes a problem [27–29]. It has been noticed that as-synthesized HAp powder does not have the ability to resist the bacterial infection since it contains only calcium and phosphate. Even though some have reported the antibacterial property of locally produced hydroxyapatite [30], there is lack of substantial evidence to prove the antibacterial property of hydroxyapatite.

In order to avoid these infections HAp ceramics need to be loaded with some external compounds that can prevent microbial colonization and show antibacterial effect to the pathogenic bacteria. Bacteria adhere on the surface of biomaterials via the surface deposited proteins and other extracellular matrixes, governs the pathogenesis of implant associated infections [31,32]. These infections cannot be completely avoided by using antibiotics due to the growth of antibiotic resistant strains of microorganisms. Even though there are many existing technologies, they are of high cost and less effective and also these antibiotics like substances will not withstand extreme processing conditions of the ceramics like high temperature and pH. This necessitates the importance of a novel and efficient method to prevent the implant associated infections. The best method to avoid infection is giving antibacterial property to the ceramics material. Among various inorganic antimicrobial agents, silver is a very attractive alternative because it possesses many advantages such as good antibacterial ability, excellent biocompatibility, and satisfactory stability. Studies have shown that Ag^+ ions are able to penetrate the bacterial cell wall and destroy the cell equilibrium.

The bactericidal activity of silver has been demonstrated since the early centuries. However studies on silver-material association for a better bacterial resistant biomaterial are comparatively a novel idea. Even though many studies have already been reported, still further studies are needed to optimize the silver amount for better property. It has been demonstrated that the higher the level of silver incorporated into a material, the better the antimicrobial effect, but it might leads to increase in cytotoxicity.

This necessitates optimizing a lower silver concentration which can cause excellent antibacterial property as well as cytocompatibility. The experiments for cell proliferation, cell cytotoxicity and cytocompatability can give a detailed picture on how the synthesized HAp–Ag composites acts with human cells upon implantation. To measure the cell proliferation and cytotoxicity, colorimetric assays such as MTT assay are recommendable due to its rapidity and precision and also no radioisotope is involved [33].

In this study, HAp ceramics are synthesized at various alkaline pH and its phase stability with respect to different calcination temperatures were studied. Further, effect of Ag addition on phase stability of HAp–Ag composite with respect to calcination temperatures was examined. Antibacterial property of HAp–Ag composite system was evaluated against *Staphylococcus aureus*. The cytocompatibility of these composites were also investigated in mouse embryonic fibroblast (NIH3T3) cells for their prospective applications in therapeutic interventions.

2. Materials and methods

2.1. Synthesis of HAp ceramics and HAp–Ag composites

The HAp ceramic powder was prepared by solution route using the analytical reagent grade calcium nitrate tetrahydrate [$Ca(NO_3)_2 \cdot 4H_2O$] (Alfa Aesar) and ammonium dihydrogen orthophosphate [$(NH_4)H_2PO_4$] (Merck) in such a way that Ca/P ratio was maintained at 1.67. Fig. 1 shows the flow chart of HAp synthesis process. First ammonium dihydrogen orthophosphate was slowly added to a solution of calcium nitrate tetrahydrate and after proper mixing, the HAp was precipitated by adding ammonia and pH of the solution was maintained from 9 to 11. The solution was stirred constantly for 24 h by mechanical stirrer, allowing the reaction to complete. The resultant precipitate was separated by centrifugation and ammonia was removed by repeated washing. The precipitate was allowed to dry in an oven at 60 °C. Subsequently, aggregates formed were crushed into fine powder and calcined at various temperatures from 800 to 1200 °C at 200 °C intervals.

In a separate study as-synthesized HAp powder was mixed with $AgNO_3$ crystals (Fischer Chem) of amounts 1–30 wt% and the resultant HAp–Ag composite powder was thoroughly mixed using a mortar and pestle for 30 min. The resultant mixed powder was calcined in the temperature range 800–1200 °C to observe the interaction of the both HAp and Ag with respect to calcinations temperatures.

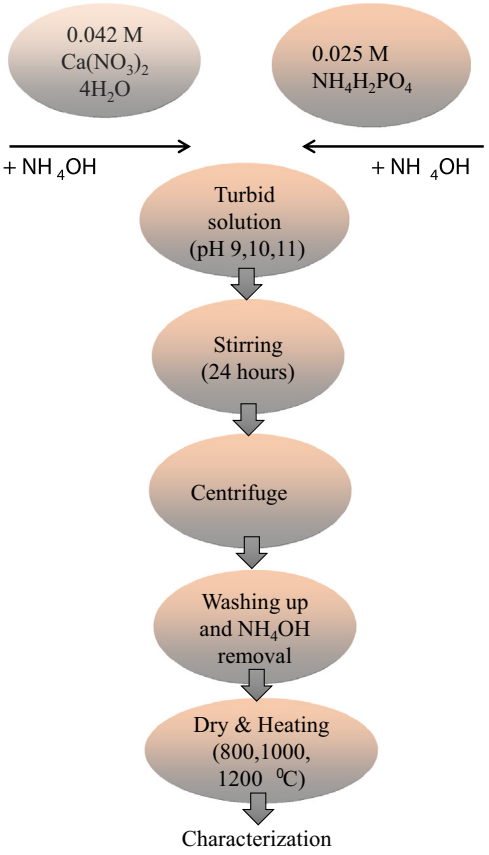

Fig. 1. Flow chart of complete HAp synthesis process.