

SILVER-IMPREGNATED HAP-COATING ON ALUMINA SUBSTRATE FOR PREVENTION OF INFECTION

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ABSTRACT

Recently ion beam assistant deposition (IBAD) was successfully used to produce a dense ultra-adherent and pinhole-free hydroxyapatite (HAp) layer on alumina substrate. After that the HAp-coated alumina was immersed in 20ppm and 100ppm AgNO₃ solution at room temperature for 48 hours to carry out the ions exchange between Ag⁺ and Ca²⁺ in HAp. The obvious antimicrobial effect against *E.Coli*, *P. Aeruginosa* and *S. Epidermidis* was observed in the samples treated with 20ppm AgNO₃ solution. In contrast to this, the untreated samples did not show any bactericidal effect. Scanning electron microscope (SEM) study showed that Ag homogeneously distributed on the surface. X-ray diffraction (XRD) demonstrated that the surface structure in the samples without Ag was HAp, whereas with Ag is HAp and AgCaP. It appears that silver ions exchange with calcium ions in HAp does not cause significant changes in the structure of HAp coatings.

1. INTRODUCTUION

Hydroxyapatite (HAp, Ca₁₀(PO₄)₆(OH)₂) has been used as implant material because of its good biocompatibility and the ability to form a strong bond to the human hard tissue. However its poor sintering properties and low fracture strength restrict its applications⁽¹⁻²⁾. Alumina (α-Al₂O₃) has high wear resistance, high strength and very stable chemical properties in the physiological environment. But alumina forms no chemical and biological bond at the material-tissue interface. Various kinds of techniques have been used to produce a HAp layer on the surface of alumina substrate⁽³⁾.

Recently ion beam assistant deposition (IBAD) is successfully used to produce a dense ultra-adherent and pinhole-free HAp layer on titanium alloy and alumina⁽⁴⁻⁵⁾. IBAD is a novel coating technology which combines physical vapor deposition with the bombardment of energetic ions⁽⁶⁻⁷⁾, through which a wide atomic intermixed zone of HAp coating and Al₂O₃ substrate was built, and then a extremely high adhesion of HAp coating to Al₂O₃ was acquired⁽⁵⁾.

Implant caused infections is considered to be a serious and common complication in orthopaedic surgery. The presence of foreign materials inside the body will not only interfere with the host's defense mechanism, but will also influence the clinical dose of a antibiotics that

is needed to protect infections. It is now recognized that a high concentration of antibiotics at the bone-implant interface would be essential to prevent these bacterial infections. Various methods have been developed for delivering antibiotics at the bone-implant interface⁽⁸⁻¹⁰⁾. In the present paper we report the method of ion exchange of Ag^+ with Ca^{2+} in HAp coatings on alumina substrate for the purpose to reduce the infections acquired by implant.

2. MATERIALS AND METHODS

2.1 COATING OF HAP ON ALUMINA BY IBAD

A polyfunctional IBAD system was used to synthesize the HAp coating. The system is mainly equipped with three broad-beam Kaufman ion sources, one rotatable water-cooled sample holder, and one rotatable target. In the experiments, the base pressure of the chamber was below 3×10^{-4} Pa, the working pressure was about 1.8×10^{-2} Pa. The flat $12\text{mm} \times 12\text{mm} \times 1.2\text{mm}$ alumina samples were ultrasonically degreased in acetone and alcohol bath each for 10 min. The substrate surfaces were cleaned by the ion beam bombardment of $100\mu\text{A}/\text{cm}^2$ with 3 keV for 10 min. HAp Target was sputtered by 3.5 keV Ar^+ , and resulting coatings were simultaneously bombarded by 200 eV Ar^+ for reinforcing the compactness. Substrate temperature measured during deposition was below 100 °C. In addition, an intermixed layer of coatings with substrates was produced by dynamic recoil mixing with 30 keV Ar^+ bombardment.

After coating, the samples were annealed at 500 °C for 2 hours and then immersed in deionized water for 72 hours at room temperature to carry on the transformation of HAp from amorphous state to crystalline. The thickness of the coating is measured to be 638 nm.

2.2 SILVER IMPREGNATION

The HAp-coated alumina samples were immersed in 20ppm AgNO_3 solution (12.7ppm Ag^+) at room temperature for 48 hours to carry on the ion exchange of Ag^+ with Ca^{2+} in HAp on the surface. After that the samples were put for antimicrobial test. For SEM and XRD experiments the samples were immersed in 100ppm (63.5ppm Ag^+) instead of 20ppm AgNO_3 solution to study the structure changes of HAp.

2.3 ANTIMICROBIAL TEST

The following microorganisms were used: *Escherichia Coli*, *Pseudomonas Aeruginosa* and *Staphylococcus Epidermidis*, among which *E. Coli* and *P. Aeruginosa* are gram negative and *S. Epidermidis* is gram positive. The bacteria were chosen because gram-negative bacteria are responsible for more than 80 % of all infections. With *E. Coli* being responsible for more infections than all other genera combined.

The samples of HAp-coated alumina with Ag were immersed into the phosphate buffer saline (PBS) solution (Table 1) at room temperature for 24hrs. The PBS solution contained about 1×10^5 cells/ml *E. Coli*, *P. Aeruginosa* and *S. Epidermidis*. 0.1ml of the treated PBS

solution was inoculated in the 20 ml LB agar plate to cultivate at 37 °C for 24hrs, after that the number of colonies was counted. All glass wares were sterilized in the autoclave at 120 °C for 30 min. before experiment.

Table 1 The compositions of the phosphate buffer saline solution.

NaCl	Na ₂ HPO ₄	NaH ₂ PO ₄	H ₂ O	pH
8.5g	2.2g	0.2g	1000ml	7.4

3 RESULTS

3.1 SEM

The morphology and structure of the coatings were examined by scanning electron microscopy (SEM) and energy dispersive spectroscopy (EDS). Figure 1 shows that no observable cracks were found on the surface of the HAp coatings. The coating is uniform and dense. X-rays of Ag on the surface demonstrates clearly that the Ag-rich phase homogeneously distributed on the surface (Figure 2). EDS analysis shows that besides the element Al from the substrate there are elements of Ca, P and Ag (Figure 3). The composition analysis by EDS showed that the Ca/P atomic ratio of the coatings with and without Ag is 1.19 and 1.46 respectively. The Ca/P ratio with Ag is much lower than the one without Ag. But the (Ca+Ag)/P ratio is 2.59, it is higher than the Ca/P ratio of 1.67 in HAp. This proved that some of the Ca²⁺ ions in HAp were replaced by Ag⁺ ions, the structure of the coating may be silver ions contained HAp - (Ca,Ag)₁₀(PO₄)₆(OH)₂. From the (Ca+Ag)/P ratio it is suggested that silver exists not only in HAp, but also in other phase.



Fig.1 SEM morphology of surface of the alumina sample coated with HAp by IBAD.

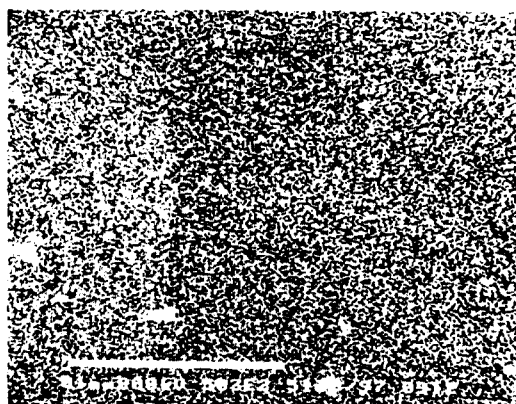


Fig.2 X-rays of Ag on the surface of sample immersed in AgNO₃ solution.

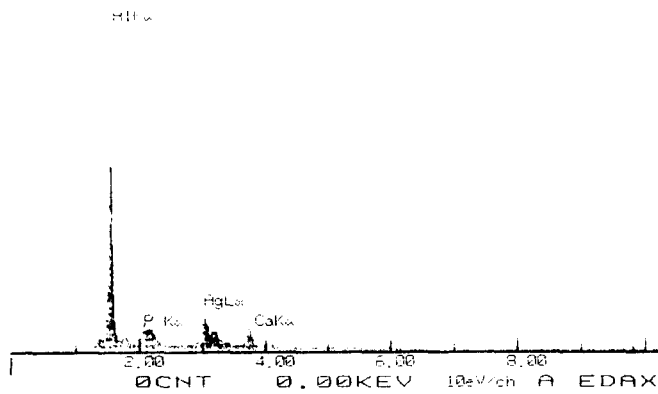
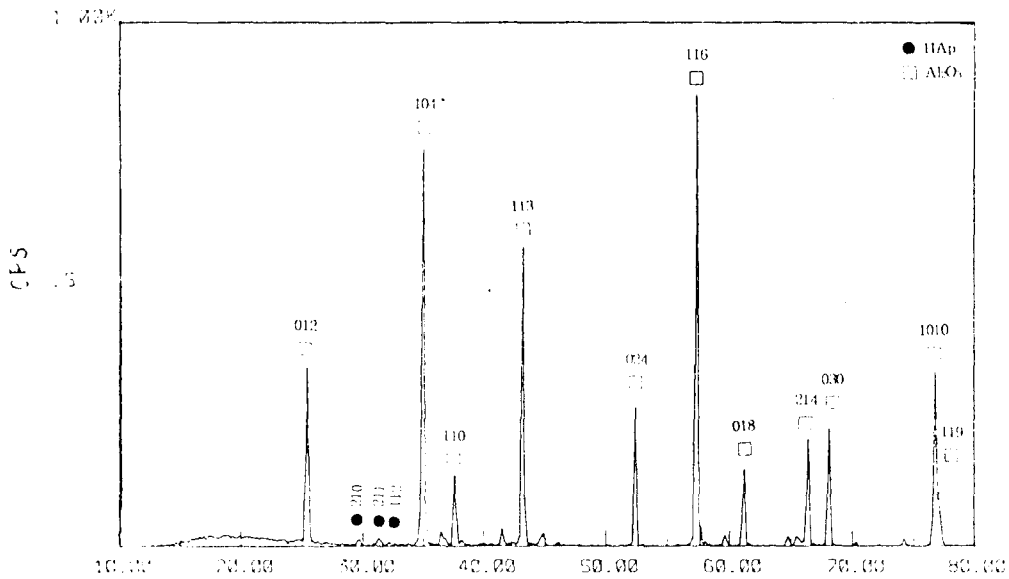


Fig. 3 EDS analysis of the sample immersed in AgNO_3 solution.

3.2 XRD

The structure of the specimens was further characterized by x-ray diffraction (XRD). Figure 4a-b is the XRD patterns from the specimens with and without Ag-treatment. The structure of the samples without Ag is alumina substrate and crystalline HAp which can be determined by the planes of (210), (211) and (112) (Figure 4(a)). The structure of the samples after Ag-immersion is shown in Figure 4(b). Besides alumina substrate and HAp there are two obvious peaks of AgCaP (001) and (111). It proved that Ag does not exist in pure form, but in the structure of HAp and AgCaP. The Ca/P ratio in HAp is 1.67, Ag/P in AgCaP is 1. EDS analysis showed that the (Ca+Ag)/P is 2.59. From which it can be confirmed that Ag presents both in HAp and AgCaP. They are consistent with the results given by SEM. It appears that silver uptake phenomenon does not cause significant changes in the structure of HAp coatings.



(a)

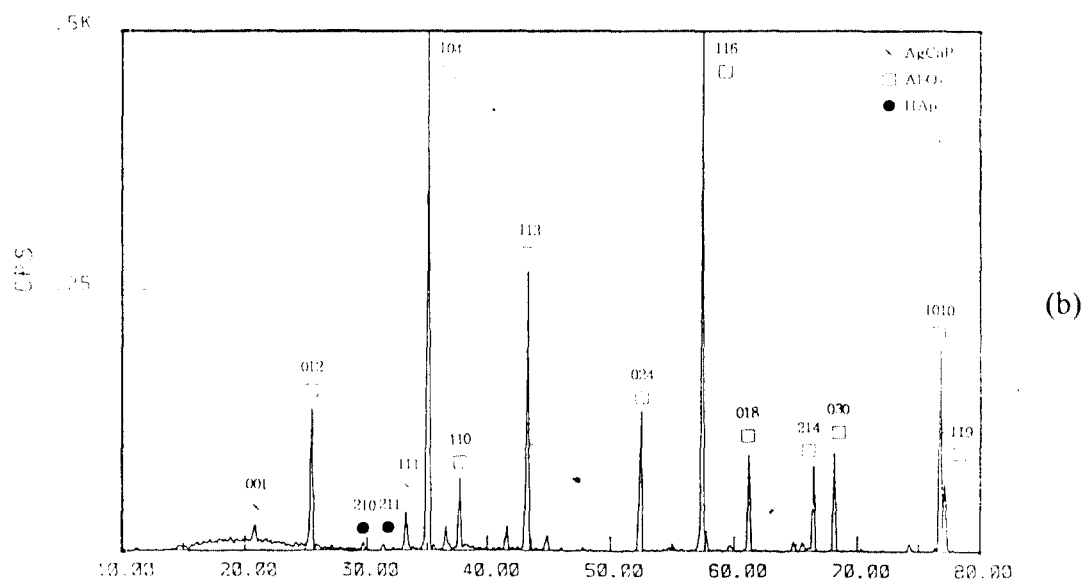


Fig. 4 XRD patterns from the samples (a) without Ag (b) with Ag .

3.3 ANTIMICROBIAL TEST

The antimicrobial effects of the specimens with and without Ag-immersion against *E. Coli*, *P. Aeruginosa* and *S. Epidermidis* are summarized in Table 2. The significant difference proved that the silver ions released from HAp have strong bactericidal effect against *E. Coli*, *P. Aeruginosa* and *S. Epidermidis*.

Table 2 Results of viable cell counts in the alumina samples coated with HAp and Ag-HAp.

samples	number of colony		
	<i>E.Coli</i>	<i>S. Epidermidis</i>	<i>P. Aeruginosa</i>
Al ₂ O ₃ +HAp	too many	too many	too many
Al ₂ O ₃ +HAp+Ag	1	33	many

4 DISCUSSION

Previous works on the antimicrobial effects of silver in vitro⁽¹¹⁻¹²⁾ have shown that silver ions have strong inhibitory and bactericidal effects as well as broad spectrum of activity. In one of our previous works, the antimicrobial ceramics were made based on HAp and composed of metal ions such as Ag⁺, Cu²⁺ and Zn²⁺. Among them the Ag⁺-HAp received the most attention for its obvious antimicrobial effect⁽¹³⁾.

In this study a very low concentration of silver ions in HAp can kill bacteria on contact and prevent bacterial colonization. The excellent bacteriostatic property of the Ag-contained HAp coating toward E. Coli is clinically significant because the microorganism is known to be responsible for more infections than all other genera combined. It is found that the material exhibited the antibacterial property toward E.Coli, S. Epidermidis and P. Aeruginosa.

M. Shirkhazadeh et.al. reported the silver ions uptake by HAp coatings in solutions and release from HAp coatings in simulated body fluid (SBF) solution(14). Silver ions will be adsorbed and immobilized to HAp coatings by an ion-exchange mechanism. Through which a bioactive delivery system was built for the slow release of antimicrobial Ag⁺ ions.

5. CONCLUSIONS

A compact crystalline HAp coating which has much higher adhesive strength to substrates has been obtained by the introduction of IBAD. Exchange of Ag⁺ ions with Ca²⁺ ions in HAp can be easily completed in aqueous solution. The Ag-impregnated HAp exhibited the best antimicrobial property toward E.Coli, followed by S. Epidermidis and P. Aeruginosa.

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