Development of Novel Inorganic Antibacterial Material

Guo-Zhong LU[1,a,*], Xiu-Juan DING[2,b], Wei-Xuan ZHAO[3], Jin-Tai HE[4], Zhan-Xiong MA[5]

[1,3,4,5] Beijing Building Materials Academy of Sciences Research, Beijing 100041, P. R. China

[2]Northeastern University at Qinhuangdao,Qinhuangdao, Hebei, China, 066004,P.R. China

[a]luguozhong@163.com, [b]dingxiujuanlove@126.com

*Corresponding author

Keywords: Antibacterial material, Ti(IV)/metal ions combined with HAP, Activity, Cooperativity.

Abstract. This paper introduced a novel and efficient inorganic antibacterial material technique, which used hydroxyapatite (HAP) as a carrier, taking the method of co-precipitation and ion-exchange to prepare Titanium/metal ions (Ag^{+}, Cu^{2+}, Zn^{2+}) and then combined with hydroxyapatite (HAPTiM). According to the XRD characterization, the optimum doping of the titanium was determined as 0.1, without changing the structure of HAP. And the materials were further characterized by UV-vis diffuse reflectance absorption spectra. Based on the antibacterial experiment on Staphylococcus aureus and Escherichia coli, the results showed that the materials had greater antibacterial activity than nano TiO_2.

Introduction

In recent years, with the continuous improvement of people's living standard, a heavy use of air conditioning, ventilation equipment, household appliances, decoration materials and office supplies, etc, which will affect indoor air quality. But most of the time we are stuck in such enclosed spaces, follow, harmful bacteria has brought big threat to our lives and our health, and indoor diseases have sprung up. Therefore, indoor air pollution has become a serious problem, in order to avoid the spread and infection of bacteria and effectively inhibit their growth, purify living environment, the development of inorganic antibacterial materials has been paid more and more attention.

In 1985, Matsunaga and his collaborators first reported the TiO_2 photo catalytic could kill bacterial in the water[1].Since then, there has been a lot of reports about antibacterial effect of TiO_2. TiO_2 appears to be a good anti-bacterial sterilization techniques, however, the regeneration of TiO_2 powder and too high lighting costs hinder the practical application of this technology. In the weak ultraviolet light, the antimicrobial activity of TiO_2 fixed with other substances is relatively low, as for the actual purification technology, there is no such effect. By detecting the concentration variation plasma membrane composition and bacteria living attenuation curve, studying the antibacterial process of TiO_2 by atomic microscopy measurements. The results show that the antibacterial process includes two steps[2,3]. First, disrupt and partially decompose the cell outer membrane, and then inner membrane. In order to achieve the above reaction, TiO_2 requires relatively strong ultraviolet light to produce various active substances. On the other hand, the antibacterial process of metal ions includes two steps. First, inorganic ions penetrate into the cell through the cell membrane. Then they make bacteria lose activity through a simple antimicrobial reaction. There is nearly no porosity protein in the cell membrane, which will make the inorganic ion lose anti-bacterial function. It is well known that microbes have resistance on the environment and inorganic antimicrobial ion in the antimicrobial conditions. Thus, in some cases, the antibacterial function of inorganic ion cannot give full play. In order to achieve a higher antibacterial activity under weak ultraviolet light conditions, such antimicrobial ions as copper and silver that was deposited on TiO_2 film have been studied. $Ca_{10}(PO_4)_6(OH)_2$ (HAP for short) with thermal catalytic and photo catalytic properties is generally used for biological ceramics, liquid chromatography absorbers and other fields[4,5]. To overcome the shortcomings of the inorganic antimicrobial materials, we use hydroxyapatite with efficient ion exchange properties and biological adsorbability as a carrier. And to modify hydroxyapatite with Ti and metal ions by co-precipitation and ion-exchange method, developing a new and efficient inorganic antibacterial material with dual antibacterial mechanism.

Experiment

Materials

P25 TiO_2 (80% anatase, 20% rutile, Degussa Corporation) photocatalyst, purity was 99.5%, specific surface area was 50±15 m^2g^{-1}, average particle size was 21 nm, particle size distribution was from 9 to 38 nm. Escherichia coli (*E.coli* DH 4α) and staphylococcus aureus (*S. aureus* ATCC 6538), Institute of Microbiology Chinese Academy of Science.Other chemical reagents were of analytical grade, water is deionized water.

The Preparation of Hydroxyapatite Combined with Metal ion and Ti

Hydroxyapatite was prepared by the coprecipitation of calcium nitrate and phosphoric acid[5-7]. And then the metal ions like Ag^+, Cu^{2+} and Zn^{2+} exchanged with HAP to prepare metal-doped hydroxyapatite HAPAg, HAPCu and HAPZn based on the ion-exchange method. HAPTi was prepared by the co-precipitation of calcium nitrate, phosphoric acid and titanium sulfate. And then the metal ions like Ag^+, Cu^{2+} and Zn^{2+} exchanged with HAPTi to prepare HAPTiAg, HAPTiCu and HAPTiZn based on the ion-exchange method. The preparation process was as follow.

First, added 500 mL distilled waterin a beaker,heating until boiling in a constant temperature mantle to remove CO_2. Then weighed 9.44 g Ca $(NO_3)_2$, which was dissolved in 400 mL distilled water without CO_2, and set on a magnetic stirrer, adding phosphoric acid of 1.64 mL with pipette, dropping ammonia with a dropper till pH value of the solution was adjusted to 9. At this time a white precipitate appeared, and the solution in an autoclave was heated at 100 ℃ for 6 hours, took out and filtrated in vacuum. Washed ammonium ions with distilled water till the pH value of the solution was close to 6. Finally ,the white precipitate was placed in a drying oven at 70 degrees, hydroxyapatite was prepared in this way.

The preparation of HAPTi was the same as above, but based on different atomic ratio of Ti/(Ti+Ca), respectively prepared X_{Ti}=0,0.1,0.3, etc. HAPAg (Cu,Zn) and HAPTiAg (Cu,Zn) was prepared by HAP, HAPTi (X_{Ti}=0.1) nano materials based on ion-exchange method. The preparation process was as follow. Each weight of HAP was 0.5 g, adding 90 mL 0.01 mol/L $AgNO_3$, $Cu(NO_3)_2$, $Zn(NO3)_2$ solution respectively, infiltrating for 24 hours. Then filtrated in vacuum. Flushing with 2000 mL distilled water. Placed in a drying oven at 70 degrees for 6 hours, HAPAg (Cu,Zn) and HAPTiAg (Cu,Zn) antimicrobial material was prepared.

The Preparation of Nickel Net Antimicrobial Films Loaded by Different Materials

Such antibacterial material of 0.2 g were uniformLy dispersed in the silica sol solution. All the suspension was loaded on the nickel net by infiltrating. Placed the nickel netin a drying oven at 70 degrees for 2 hours to prepare antimicrobial films which was loaded by different materials , using for a activity evaluation of antimicrobial materials. The diameter of nickel net was 64 mm.

Results and Discussions

The Elemental Composition of Hydroxyapatite Combined with Titanium and Metal Ion

The determination of Ca and Ti in combined hydroxyapatite by Atomic Absorption Spectrometry was shown in Table 1.

Tab. 1 The elemental composition of HAPTiM

Sample Name	Ca Content, mmol/g	Ti Content, mmol/g	metal Content, mmol/g
HAPTi	8.91	1.04	
HAPTiAg	5.18	1.06	1.36
HAPTiCu	7.39	1.11	0.96
HAPTiZn	6.82	1.05	0.77

Table 1 showed that the atomic ratio of Ti (Ⅳ) and Ca (Ⅱ) in HAPTi was about 1:9, approximately the mixing amount of Ti (Ⅳ) (X_{Ti}=0.1), showed that Ti (Ⅳ) substituted Ca (Ⅱ), which meant that in the structure of hydroxyapatite,Ti (Ⅳ)existed in the form of divalent ions $[Ti(OH)_2]^{2+}$ and created oxygen vacancies. Zn^{2+}, Cu^{2+}, Ag^+, Ti (Ⅳ) content did not change, while the content of Ca (Ⅱ) decreased, which showed that these ions mainly substituted the Ca (Ⅱ) in HAPTi.

The XRD Characterization

The XRD spectra that varied with different X_{Ti} was shown in Fig.1. X_{Ti}=Ti/(Ca+Ti).

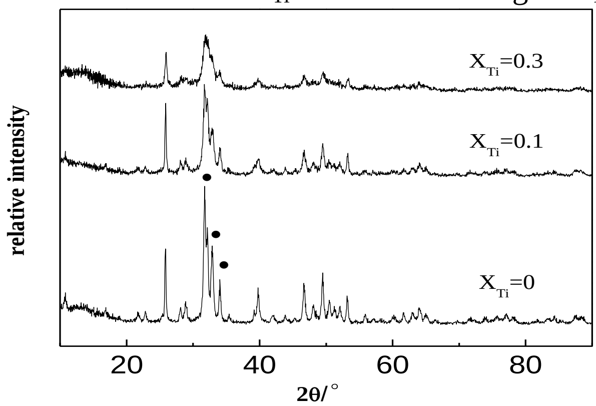

Fig.1 The XRD spectra of hydroxyapatite combined with Titanium

As was shown in Fig.1, the peak strength decreased while the X_{Ti} value increased. When X_{Ti}=0.3, the peak strength decreased significantly, which showed that the doping content of Ti increased with lower crystallinity of HAP. Compared with characteristic diffraction peaks of HAP, there was significant crystal structure peak as X_{Ti} =0.1. As a result, X_{Ti}=0.1 has been decided.

In this experiment, we got HAPTiM by ion exchange (HAPTi ,X_{Ti}=0.1), where, M is Zn^{2+}, Cu^{2+} or Ag^+. The XRD spectra was shown in Fig.2.

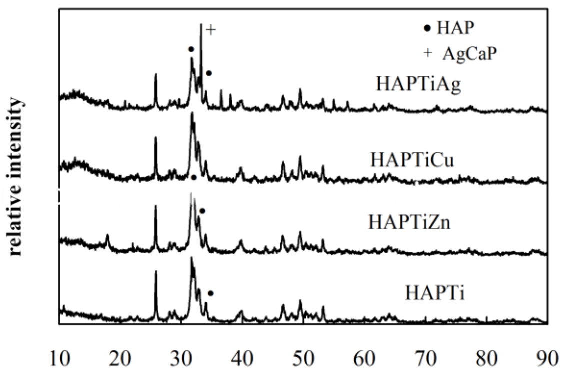

Fig.2 The XRD spectra of hydroxyapatite combined with metal ions

Fig.2 showed that the exchanged Zn^{2+}, Cu^{2+}, and Ag^{+} did not significantly affect the crystal structure, and there was no obvious peak of copper and zinc appeard. However, in addition to the hydroxyapatite diffraction peaks, there was a clear peak of AgCaP at 2θ=33.4°.

The UV-vis Characterization

The UV-vis spectrogram of P25 TiO_2 and HAPTi was shown in Fig.3.

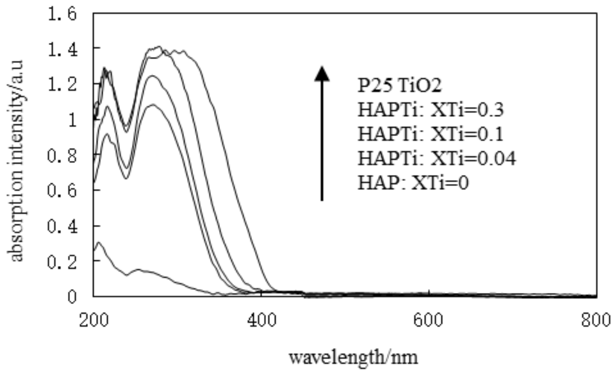

Fig.3 The UV-vis spectrogram of P25 TiO_2 and HAPTi with different X_{Ti}

As was shown in Fig.3, the UV absorption of sample X_{Ti}=0.1 and X_{Ti}=0.3 was above 370 nm, but there was no in modified HAP spectrogram. While the modified HAP showed a main absorption peak approximately at 370 nm, which was similar to the UV absorption of P25 TiO_2[6]. This showed that the Ti (Ⅳ) had changed surface properties of HAP particles. However, the calcium ion of hydroxyapatite was gradually substituted by Ti^{4+} with the increase of X_{Ti}, the absorbance of the sample increased to the level as TiO_2. These results indicated that Ti (Ⅳ) incorporated into the structure of HAP.

The UV-vis spectrograms of HAPTi combined with different metal ions were shown in Fig.4.

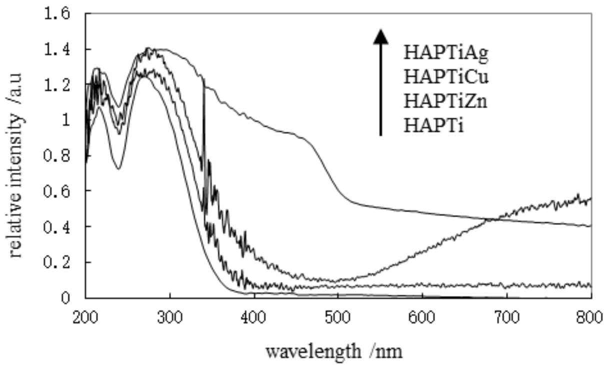

Fig.4 The UV-vis spectrogram of HAPTi and HAPTiM

The UV-vis spectrograms of HAPTi combined with different metal ions were different in Fig.4. There was a new absorption peak in the spectral range of HAPTiAg at 400-500 nm, HAPTiCu at 500-800 nm. However, there was no in the spectral range of HAPTiZn.

Study on Antibacterial Activity of Antibacterial Materials under Different Conditions

Currently, there are a variety of evaluation methods in the antibacterial activity of antibacterial materials, common methods are inhibition zone method, dynamic testing method and plate-counter method. According to the performance characteristics of the prepared material, plate-counter method has been adopted. Light reaction, dark reaction of series of antibacterial materials and the antimicrobial activity of Escherichia coli (E. coli) and Staphylococcus aureus (SA) in different light intensity was evaluated, compared with the P25 TiO_2. Before the evaluation of bactericidal activity , beef extract-peptone, LB medium, 0.9mL normal saline, several centrifuge tubes , mediums and some tubes with normal saline should be prepared, the pipette tips wrapped in newspaper can be sterilized by steam under high pressure. The pressure, temperature, time of sterilizing pot is 0.1 Mpa, 121.5 ℃, 20 min. Before the experiment, bacteria should be cultured in advance for 18 hours in a constant temperature shaker, the activity of the bacteria reached the maximum. Antibacterial materials loaded by nickel mesh were sterilized under ultraviolet lamp for 10 min. The light intensity of the UV lamp was 0.2 mw/cm^2(365-UV), the initial concentration of bacteria was 5×106 CFU/mL.

Fig.5 indicated that the influence of nickel mesh loaded by HAPTi,HAPTiM and P25 TiO_2 on survival of Escherichia coli under weak ultraviolet lamp (0.2 mw/cm^2 uv-365nm) with the reaction time. Obviously, the survival rate of Escherichia coli on the P25 TiO_2 film did not change under the dark and light conditions, HAPTi film had less change under light conditions. The results indicated that killing gram-negative E. coli need relatively strong ultraviolet light by photocatalytic. Instead, the bacterial survival rate of HAPTiM film reduced even in the dark, and faster than HAPTi film under light conditions, which proved HAPTi combined with Ag^+, Cu^{2+}, Zn^{2+} had antibacterial function in the dark. In this experiment, HAPTiM film released metal ion into solution was proved by atom absorption spectra. When the HAPTiM film was irradiated by 0.2 mw/cm^2 (uv-365nm) UV, there was less E. coli survival than in the dark, the bactericidal function of HAPTiM was much higher than P25 TiO_2 and HAPTi in the weak UV. There is a synergistic effect of HAPTiM between photocatalytic reactions and metal antimicrobial reactions under light conditions.

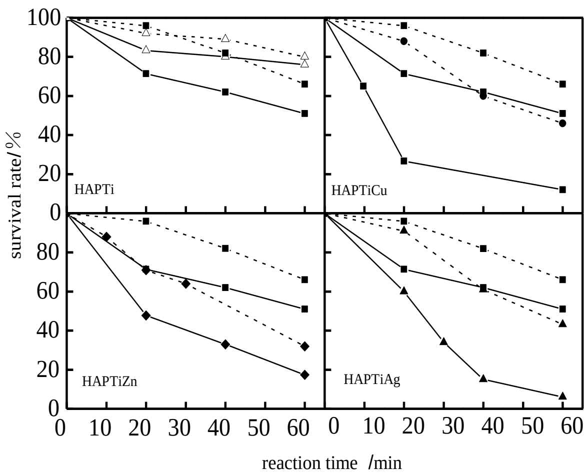

Fig.5 The influence of nickel mesh loaded by HAPTi,HAPTiM and P25 TiO_2 on survival of Escherichia coli under weak ultraviolet lamp

In Fig.5, HAPTi film (■), HAPTiZn film (◆), HAPTiCu film (●), HAPTiAg film (▲), P25 TiO_2 film (△) (The solid line is the photoreaction reaction, the dashed line is the dark reaction).

Study on Antibacterial Activity of Staphylococcus Aureus

Fig.6 indicated that the influence of different antibacterial materials on survival of staphylococcus aureus under weak ultraviolet lamp with the reaction time. Compared with Fig.5, it can be observed that the inactivation of staphylococcus aureus had a significantly different, P25 TiO_2 and HAPTi under light conditions, the survival rate of staphylococcus aureus dropped faster than E. coli. The results showed that Gram-positive bacteria staphylococcus aureus was easier to kill than gram-negative bacterium E. coli. For these two bacteria, HAPTi showed higher activity than P25 TiO_2, staphylococcus aureus survival rate decreased 7.7%. It was determined that HAP had exhibited a good affinity to biological materials. Thus, the membrane surface of HAPTi and HAPTiM adsorbed more cells, another advantage of the material was to improve the bactericidal activity. Further, in the dark, HAPTiM for killing S. aureus showed higher antimicrobial activity than E. coli, these results also showed that the Gram-negative bacterium E. coli had more resistant to external hazards. Similarly, under light conditions, HAPTiM film enhanced the inactivation of S. aureus, these results also showed that there is a synergistic effect of HAPTiM between Ti (Ⅳ) photocatalytic reactions and antibacterial ions to kill staphylococcus aureus.

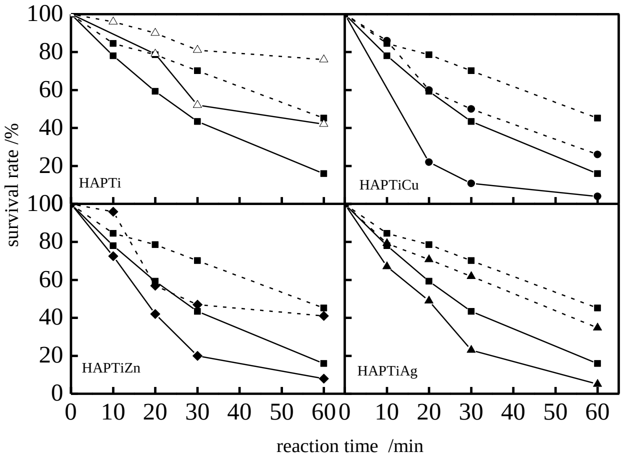

Fig.6 The influence of different antibacterial materials on survival of staphylococcus aureus under weak ultraviolet lamp with the reaction time

In Fig.6, HAPTi film (■), HAPTiZn film (◆), HAPTiCu film (●), HAPTiAg film (▲), P25 TiO_2 film (△) (The solid line is the photoreaction reaction, the dashed line is the dark reaction).

The results showed that during the antibacterial process of HAPTiAg and HAPTiCu, there is a synergistic effect of Ti (Ⅳ) and Ag^+ (Cu^{2+}) between oxidation and antibacterial reaction. On the one hand, first, cell outer membrane was damaged by $O_2^{\bullet -}$ which was produced by HAPTiAg (Cu). Ag^+ (Cu^{2+}) got into the interior of the cell through the damaged cell membrane. Finally, Ag^+ (Cu^{2+}) made bacteria inactivation by antibacterial effect. On the other hand, the inhibitory effect of ions enhanced the sterilization efficiency of $O_2^{\bullet -}$.

Conclusion

Through the characterization of HAPTiM and study on antibacterial activity, the results are as follows.

(1) Analysis the elemental composition of HAPTiM antibacterial material by atomic absorption spectrometry, it was found that titanium and metal ions mainly substituted HAP in Ca (Ⅱ).

(2) The best doping content of titanium that did not change the structure of HAP , X_{Ti}=0.1 has been decided according to XRD characterization. Adding Zn^{2+}, Cu^{2+}, Ag^+ did not reduce the crystallinity of HAPTi.

(3) In the weak ultraviolet light or dark conditions, HAPTiM for killing E. coli and S. aureus showed higher antimicrobial activity, and their antibacterial activity was much higher than the P25-TiO_2 film.

(4) HAPTi,HAPTiAg (Cu) can produce O2•− under light or dark conditions, but HAP,P25 TiO2 and HAPTiZn can only produce O2•− under light conditions. The injury mechanism of cell was the synergistic effect of O2•− between oxidation and antibacterial reaction.

References

[1] Matsunaga T. Tomda R. Pholoelctrochemical Slerilization of mierobial Cells by semiconductor Powders [J]. FEMS Microbial Lett.1985,29:211.

[2] Tanizawa, Y.; Sawamura, K.; Suzuki, T. Reaction characteristics of dental and synthetic apatites with Al^{3+} and La^{3+} ions in acidic solutions. J. Chem. Soc. Faraday Trans. 1990, (86):4025-4030.

[3] Chun Hu, Yizhong Wang, Hongxiao Tang. The theory and practice progress of heterogeneous photocatalysis. Advance in Environmental Science. 1995,3(1):55-64.

[4] Zhiqing Kuang, Chunyyue Pan, et al. The preparation of hydroxyapatiteultrafine powder. Journal of Central South University. 2000, 31(3): 246-248.

[5] Sawyer, D.T.; Valentine, J. S. How super is superoxide. Acc Chem. Res. 1981, (14):393-400.

[6] Ma, W.; Huang, Y.; Li, J.; Chen, M.; Song, W.; Zhao, J. An efficient approach for the photodegradation of organic pollutants by immobilized iron ions at neutral pHs. Chem. Commun. 2003, 1582-1583.

[7] Miyake, M.; Ishigaki, K.; Suzuki, T. Structure refinements of Pb^{2+} ion-exchanged apatites by x-ray powder pattern-fitting. J. Solid State Chem. 1986, 61, 230-235.