**BME0006**

# Plastic-strain induced synthesis of fluorescent hydroxyapatite complex and its biomedical applications for antibacterial coating

Morakul S.[1], Otsuka Y.[2,*], Ohnuma K.[3], Tagaya M.[1], Mototsuka S.[4], Miyashita Y[5] and Mutoh Y.[2]

[1]Nagaoka University of Technology, Materials Science Engineering, 1603-1 Kamitomioka, Nagaoka, Niigata 940–2188, Japan
[2]Nagaoka University of Technology, department of System Safety, 1603-1 Kamitomioka, Nagaoka, Niigata 940–2188, Japan
[3]Nagaoka University of Technology, department of Bioengineering, 1603-1 Kamitomioka, Nagaoka, Niigata 940–2188, Japan
[4]Gifu National College of Technology, Mechanical engineering, 2236-2 Kamimakuwa Motosu Gifu 501–0495 Japan
[5]Nagaoka University of Technology, Mechanical Engineering, 1603-1 Kamitomioka, Nagaoka, Niigata 940–2188, Japan

***Abstract***

In order to suppress the bacterial infection in implant applications, this study proposes development of new complex coating that enhances antibacterial properties. In present investigation we will discuss forming a fluorescent complex between HAp coatings on Ti6Al4V substrate with ligands of amino acid by using Cold Isostatic Pressing (CIP) process. Consideration of chemical behavior on mechanical stress and shear force between solids particles, three amino acids; phenylalanine, tryptophan and tyrosine were used in the CIP process. By applying pressures from 200 MPa to 800 MPa, fluorescence of HAp with the three amino acids was successfully observed by UV irradiations and fluorescent microscope. In highly compressed samples, wavelength of fluorescence were slightly shifted to longer length.

***Keywords:*** Hydroxyapatite, Amino acid and complex fluorescent coating

## 1. Introduction

Recently, Hydroxyapatite-coated titanium alloys becomes widely used in biomaterial because of its high strength and corrosion resistance. Hydroxyapatite has excellent osteoconductivity and biocompatibility. However, in dental implants application has been increasing in revisions due to the infections occur [1]. There has been widely investigated about silver ion doping or $TiO_2$ nanotube as antibacterial ligands [2]. Unfortunately, such the conventional antibacterial ligands also suffer human cells and their antibacterial performances are extremely difficult to be controlled. There are many researchers want to suppress bacterial infections on HAp coating by surface chemical treatment such as TiO2 nanotube anodization. Their results demonstrated that nanometer surface can enhance fibronectin absorption and possibly decreases bacterial adhesion. However, osteoblast cell proliferation was not improved. [3] Both consideration of wettability and chemical surface treatment were discussed by using silver doped on HAp surface. Results showed that both cell and bacterial were removed due to releasing of silver ion which it becomes non biocompatibility.[4,5] Therefore, it is necessary to develop antibacterial coating as well as biocompatible one in order to suppress bacterial infection. In this study, complex HAp coating on Titanium alloys implants that enhance biocompatibility and antibacterial property is necessary. Our group previously developed HAp fluorescent complex by CIP [6]. The complex of HAp with photocatalyst thermal-sprayed coating was exhibited an enhanced antibacterial property. The ligands of HAp complex have cytotoxicity and then it should be replaced by biocompatible one. However, the fluorescent density and contribution are not uniformly. It is necessary to investigate the photoluminescent spectrum and effect of CIP pressurized compressive force on fluorescent formation.

This study aims at forming a complex of HAp with amino acid by using CIP process in order to suppress the cytotoxicity. Present results demonstrate that the effect of pressurize compressive force from CIP affects to fluorescent density and CIP is success in fabricate complex formation between HAp and Amino acid.

## 2. Experimental procedure

### Fabrication of HAp coating and HAp fluorescent complex by CIP process

Ti6Al4V substrate was made in rectangular shape with dimension 50 mm×10 mm×3 mm. HAp powder 100% wt was deposited by plasma-spray on Ti6Al4V alloys substrate. In order to obtain luminescent complex HAp coating surface, 500 mg of three types ligands of protein amino acid; Phenylalanine, Tryptophan, and Tyrosine (Kishida Chemical Co. Ltd., Osaka, Japan), were put on the HAp coating surfaces and sealed in plastic bags by using vacuum drawing machine. Cold isostatic pressing (CIP) process (Model P-500, Kobe Steel, Ltd., Japan) with pressurized condition at 200, 400, 600 and 800 MPa holding for 20 minutes were applied on the samples. After the CIP process, fluorescence properties of surface were observed by ultraviolet light with excitation wavelength 315-400 nm (FPL27BLB, Sankyo Denki Co., Ltd.) The fluorescent surfaces were taken by

# BME0006

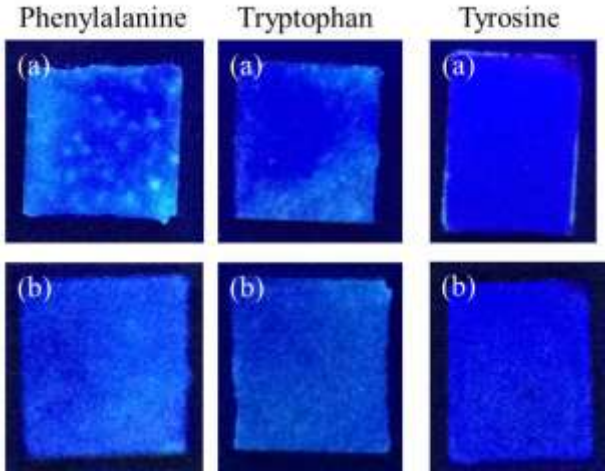


Fig.1 a) fluorescence after CIP of complex HAp coating b) complex HAp coating after 2 months immersion

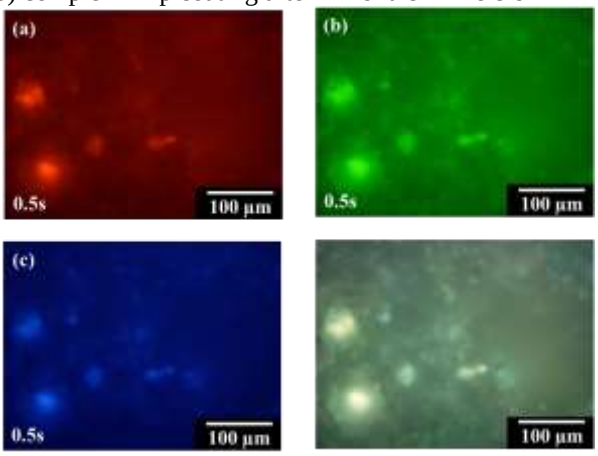


Fig.2 Effect of CIP at 800 MPa on fluorescent spectra of HAp/Tryptophan complex (a) fluorescent surface excited by UV light (bandpass filter was 460 ± 20 nm), (b fluorescent surface excited by blue light (bandpass filter was 535 ± 25 nm), (c) fluorescent surface excited by green light (bandpass filter was 605 ± 27.5 nm).

luminescence microscope (BZ-8100, Keyence Co., Ltd., Japan.) The excitation wavelength from blue, green and red light conditions are as following; 360 ± 20 nm, 470 ± 20 nm and 540 ± 12.5 nm with exposure time 0.5 s. In this BZ-8100 machine uses detection filter; 460 ± 20 nm, 535 ± 25 nm and 605 ± 27.5 nm, respectively. Dissolving of fluorescence complex coating was performed in immersion testing and remained fluorescence was observed before and after 2 months DI water immersion.

### 3. Results and discussions

CIP process is successfully established complexation between amino acid and HAp coating. This phenomenon was affected by plastic strain force that induced between solid particles.

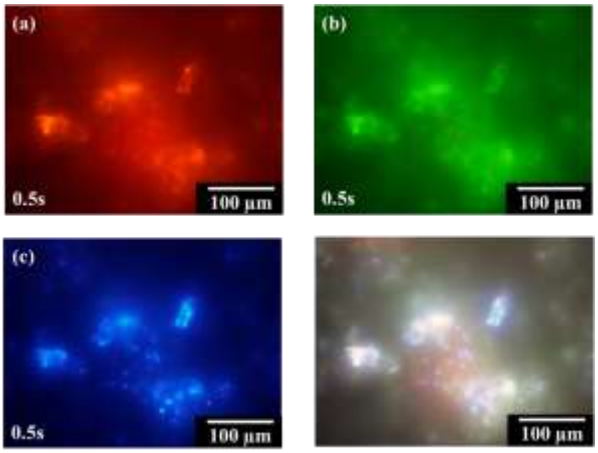


Fig.3 Effect of CIP at 800 MPa on fluorescent spectra of HAp/Phenylalanine complex (a) fluorescent surface excited by UV light (bandpass filter was 460 ± 20 nm), (b fluorescent surface excited by blue light (bandpass filter was 535 ± 25 nm), (c) fluorescent surface excited by green light (bandpass filter was 605 ± 27.5 nm).

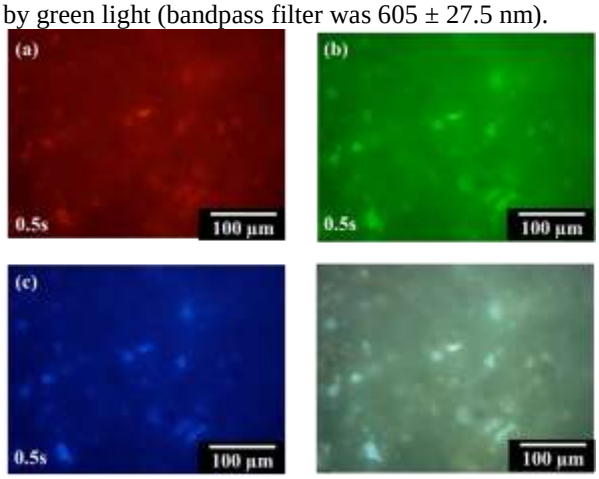


Fig.4 Effect of CIP at 800 MPa on fluorescent spectra of HAp/Tyrosine complex (a) fluorescent surface excited by UV light (bandpass filter was 460 ± 20 nm), (b fluorescent surface excited by blue light (bandpass filter was 535 ± 25 nm), (c) fluorescent surface excited by green light (bandpass filter was 605 ± 27.5 nm).

During applying compressive force, mechanochemical reaction is occurred between solid HAp coating surface and amino acid solid powder particles. The surface shows luminescence by UV irradiation. Figure 1 demonstrates remaining fluorescent after immersion in water environment although the luminescence becomes weaker during immersion. In figure 2, 3, and 4 showed the fluorescent observation by using fluorescent microscope where coating surface was excited by UV light, blue light and green light while bandpass filters were 460 ± 20 nm, 535 ± 25 nm and 605 ± 27.5 nm respectively. The red shifted of fluorescent spectra is occurred, especially in case of phenylalanine /HAp complex at highly compressive force of 800 MPa during CIP process. This behavior is consistent with the result reported by Matsuya et. al.. [6]

# BME0006

## 4. Summary

In order to obtain fluorescent complex HAp/Amino acid coating for implants, CIP process was conducted and success in fabrication of fluorescent complex coating.

1. CIP process is able to form fluorescent complex between HAp coating and amino acid.
2. Long period immersion testing revealed that there are no effects of metal ion releasing because fluorescent still remained on the surface. It is expected to be able working in human body fluid.

Future work, the fluorescent spectrum will be investigated in order to discuss the effect of plastic strain induce fluorescent complex HAp/amino acid coating formation from various pressure in CIP process. Application of photocatalyst composite HAp/$Ti_2O_3$ complex amino acid fluorescent coating will be considered in the enhancement of antibacterial properties after visible light irradiation.

## 5. References

[1] B.R. Chrcanovic, T. Albrektsson, A. Wennerberg, *Journal of dentistry 42 (2014) 1509-1527*

[2] D. Bociaga, P. Komorowski, D. Batory, W. Szymanski, A. Olejnik, K. Jastrzebski, W. Jakubowski, *applied surface science 355 (2015) 388-397*

[3] C.C. Elias, Y. Oshida, J.H.C. Lima, C.A. Muller, *Journal of the mechanical behavior of biomaterials I (2008) 234-242*

[4] D. Bociaga, P. Komorowski, D. Batory, W. Szymanski, A. Olejnik, K. Jastrzebski, W. Jakubowski , *applied surface science 355 (2015) 388-397*

[5] C. Albers, W. Hofstetter, K. Siebenrock, R. Landmann and F. Klenke, *The bone and joint journal*

[6] T. Matsuya, Y. Otsuka, M. Tagaya, S. Motozuka, K. Ohnuma, Y. Mutoh, *Materials Science and Engineering C 58 (2016) 127–132*