Journal of the Mechanical Behavior of Biomedical Materials 88 (2018) 406–414

Contents lists available at ScienceDirect

Journal of the Mechanical Behavior of Biomedical Materials

journal homepage: www.elsevier.com/locate/jmbbm

Effects of compression on orientation of ligands in fluorescent complexes between hydroxyapatite with amino acids and their optical properties

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ARTICLE INFO

Keywords:
HAp– amino acid complex
Fluorescent materials
Raman spectra
Polarized Raman
Scanning probe microscopy

ABSTRACT

This study aims to reveal the effects of pressure during cold isostatic pressing (CIP) on the microstructure and optical properties of fluorescent HAp complexes. Although the microsturucture-dependent properties of fluorescent HAp complexes have been reported to improve the antibacterial properties of photocatalyst coating layers, the mechanism behind the changes in the fluorescence properties of highly compressed HAp complexes has not yet been unveiled. CIP was successfully used to fabricate fluorescent HAp – amino acid complexes, and their fluorescence intensities increased with increasing fabrication pressure. Peak wavelength of fluorescence emitted by the HAp – amino acid complexes exhibited yellow to red shift. Although the thickness of the amino acid layer was saturated in higher pressure cases, the concentration of amino acids increased proportionally with pressure, which suggests changes in the packing structures of the ligands in the HAp– amino acid complexes. Polarized Raman spectroscopy measurements clearly detected ligands normally arranged to the HAp layer under high pressure fabrication conditions, which can provide the tightly packed ligand structure in the HAp– amino acid complexes. These tightly packed ligand structure in the HAp– amino acid complexes could emit stronger fluorescence owing to the increased density of complexations. This newly found pressure dependency in the optical properties of HAp–amino acid complexes is beneficial for developing biocompatible fluorescence materials or enhancement agents for antibacterial coating layers.

1. Introduction

Titanium alloys are widely used for artificial hip joints and dental implants because of their high mechanical strength and corrosion resistance (Niinomi, 1998; Sun et al., 2001). In order to bond titanium alloys implants to bone, hydroxyapatite (HAp) coating layers are normally applied as the biocompatible coating owing to its excellent osteoconductivity (Sun et al., 2001; Palm et al., 2002; ReikerÅs and Gunderson, 2006). HAp coating layers have successfully been deposited by plasma-spraying on the surface of Ti-6A-l4V alloys for dental implants (Heimann, 2006). However, after long-term use, the need for implant revision increases owing to bacterial infection (Drake et al., 1999; Chrcanovic et al., 2014; Gupta et al., 2011; Quirynen et al., 2002). Especially for dental implants, inhibiting the attachment of biofilms on the surfaces of the dental implants is indispensable for preventing bacterial infection (Drake et al., 1999; Subramani et al., 2009; Bruellhoff et al., 2010; Hansen et al., 2012). Researchers have tried depositing silver ions or TiO_2 nanotubes on the surfaces of implants in order to suppress bacterial infections (Chen et al., 2006; Fielding et al., 2012; Trujillo et al., 2012; Shimazaki et al., 2010; Lim et al., 2013; Bociaga et al., 2015; Cochis et al., 2016; Yanovska et al., 2014; Tian et al., 2016; Xiong et al., 2017). Unfortunately, such conventional antibacterial agents also suffer human cells because of difficulty in controlling the concentration of the antibacterial agents. Therefore, the development of antibacterial and biocompatible coating layers is important for suppressing infection in dental implants (Swartjes et al., 2015; Goodman et al., 2013; Raphel et al., 2016). Researchers have proposed several methods for modifying surface charges

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https://doi.org/10.1016/j.jmbbm.2018.09.006
Received 19 June 2018; Received in revised form 3 September 2018; Accepted 3 September 2018
Available online 05 September 2018
1751-6161/

by chemical treatments (Gottenbos et al., 2003; Lim et al., 2014) or adding peptides/protein (Harris et al., 2004; Zhang et al., 2008; Chen et al., 2017) to fabricate such the multifunctional coating layers. Although these coating layers inhibit bacterial attachment on the surfaces of implants, their antibacterial properties are hard to control because of the absence of antibacterial agents.

Fluorescent complexes of HAp combined with photocatalysts have recently been proposed as enhancement agents for the controllability of antibacterial properties (Matsuya et al., 2016, 2018; Morakul et al., 2016). This technology was biologically inspired by the light-condensing effect of fluorescence in mesophotic reefs at the depth of 50–60 m in seawater, which increases the efficiency of photosynthesis (Eyal et al., 2015). In the case of a fluorescent complex of HAp with 8-hydroxyquinoline (8Hq), differing amounts of pressure during the fabrication process could provide changes in fluorescence intensity and peak wavelength (Matsuya et al., 2016). Furthermore, the surface charges on the surface of HAp coating layers were stably changed by ligands in the HAp complexes, which affected their antibacterial performance (Morakul et al., 2016). Although the microsturucture-dependent properties of HAp fluorescent complexes improved the antibacterial property of a photocatalyst coating layer (Matsuya et al., 2018; Morakul et al., 2016), the mechanism behind the changes in the fluorescence of highly compressed HAp complexes has not yet been unveiled.

This study aims to reveal the effects of pressure during cold isostatic pressing (CIP) on the microstructure of fluorescent HAp complexes and their optical properties. Firstly, the fluorescence wavelengths of different types of HAp complexes were observed by photoluminescence (PL). Raman spectroscopy was conducted for both pure powder and fluorescent complexes of HAp with amino acids in order to discuss the concentration of ligands. Raman spectra can reveal residual stress (Nimkerdphol et al., 2014), the formation of hydrogen bonding (Zhao et al., 2016; Karakaya et al., 2015), or dimers and phase changes (Myshakina et al., 2008). An effect of mechanical loading on the peaks shift in the Raman spectra of amino acid ligands has been observed by in situ Raman spectroscopy as well as four-points bending(4PB) (Nimkerdphol et al., 2014). Polarized Raman spectroscopy was also conducted to discuss the orientation of the fluorescent complexes of HAp with amino acid coating layers. The relationships between changes in the microstructure of the HAp complexes and their optical properties were discussed.

2. Experimental procedure

2.1. Preparation of specimens

Pure powders of three types of amino acid—phenylalanine (Phe), tryptophan (Trp), and tyrosine (Tyr) (Kishida Chemical Co., Ltd., Osaka, Japan)—were used. Rectangular silicone molds were filled with these powders (KE-17; Shin-Etsu Chemical Co., Ltd., Tokyo, Japan). The silicone molds were tightly wrapped with plastic wrap and put into a vacuum incubator for 1 h. The molds were finally vacuum-sealed in plastic bags (AS-V-01, Kyutarou; Asahi Industry Co., Ltd., Nagoya, Japan). The molds were pressurized using a CIP machine (Model P-500; Kobe Steel, Ltd, Japan) at 200, 400, 600, 800 MPa for holding times of 20 min each. These pressurizing conditions were referenced from previous studies (Matsuya et al., 2016; Morakul et al., 2016).

Fluorescent complexes of HAp with the three types of amino acids were fabricated by using the CIP process. HAp powders and amino acids powders were mixed in a ball mill at weight ratios of 1:1. The mixed powders were then dried in a vacuum chamber for 1 h, and vacuum-sealed in plastic bags. The sealed plastic bags were subsequently placed in the vacuum chamber again for 24 h. The sealed plastic bags were pressurized using the CIP machine with the same pressurizing conditions.

The same CIP procedure was applied to sealed amino acid powders on plasma-sprayed HAp / Titania coating layers on Ti-6Al-4V substrates with dimensions of 50 mm × 10 mm × 3 mm. For 4PB in Raman spectroscopy, amino acid powders were adhered on a surface of Ti-6AL-4V substrates with dimensions of 35 mm × 5 mm × 3 mm. The adhesive used was cyanoacrylate base cement (CC-33A; Kyowa Co., Ltd., Osaka, Japan). The side surfaces of the amino acids layers were polished using # 1200 emery paper. A strain gage (KFG-2–120-C1-11L1M2R; Kyowa Co., Ltd., Osaka, Japan) was adhered on the compression side of the substrates.

2.2. Optical property measurements

Fluorescence from HAp– amino acid complexes was observed by fluorescent microscope (BZ-8100, Keyence Co., Ltd.). The excitation source were 360 ± 20 nm, 470 ± 20 nm and 540 ± 12.5 nm respectively with exposure time for 0.5 s. The filtering diffraction of light was DF and MF40. The luminescent results were observed in blue(460 ± 20 nm), green(535 ± 25 nm) and red (605 ± 27.5 nm) fluorescence. Photoluminescence(PL) spectra was also observed using Fluorescence Spectrophotometer (F-7000, Hitachi High Technology, Japan). Photoluminescence excitation (PLE) and PL spectra were measured from 200 nm to 800 nm using the sampling interval of 10 nm, scanning rate of 30,000 nm/min, excitation slit of 5 nm, emission slit of 20 nm and photomultiplier voltage of 400 V, respectively. UV - filter was inserted at the emission side in order to cut the fluorescence emitted by amino acids whose maximum wavelength was in the UV range (290 nm (Phe),305 nm(Tyr),and 350 nm(Trp), respectively) (Wünsch et al., 2015). Though the HAp powders reflected the excitation light, the PL intensity was calculated using the background subtraction of the intensity of HAp powders from the one of HAp-amino acid complexes. PL spectra were fitted into Gaussian function ($a\exp(-\frac{(x-b)^2}{2c^2})$;indeterminate coefficients; a, b, c) using a non-linear least-squares method solved by Gauss - Newton method in R 3.4.2 package. Quantum yields of fluorescence by HAp – amino acid complexes were also measured using absolute PL quantum yields measurement system (Quantaurus-QY, Hamamatsu Photonics, Japan).

2.3. Surface morphology measured by scanning probe microscope(SPM)

In order to evaluate the thickness of the ligand layers of the HAp–amino acid complexes, SPM measurements were conducted. Micro-Vickers hardness tests were first performed on the surface of the fluorescent HAp–amino acid complex layers to expose the HAp coating layers at the edges of Vickers markers. Contact mode measurements were performed using a micro-cantilever (OMCL-TR800PSA-1, Olympus) with the conditions of the laser operating point at 0.2 V, P-gain at 0.001 and I-gain at 700.0 (SPM-9700, Shimadzu Co. ltd.). The thickness of the ligand layers was then measured in steps by surface profiles from SPM measurements.

2.4. Raman spectroscopy measurements

Raman spectroscopy measurements were conducted to identify structural changes in the fluorescent complexes of HAp with amino acid powders. Twenty measurements were accumulated with a laser source of 532 nm (25 mW),grating of #2400, magnification of ×100, and exposure time of 4 s (LABRAM HR 800, Horiba Jobin Yvon). For mechanical loading tests, a 4PB jig (Nimkerdphol et al., 2014) was placed under the camera of the Raman spectroscopy instrument (LABRAM HR 800, Horiba Jobin Yvon). The load step was 10 N, and Raman spectra at the maximum bending stress point were observed every 10 N until the specimen was broken. The applied load was converted into bending stress, σ, using the elastic formula of 4PB. Though the fluorescence effect was relatively low, only the sample of HAp-Phe complex was used for the measurement.

In order to suppress fluorescence during the Raman spectroscopy