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Laser-assisted biomimetic process for surface functionalization of titanium metal

Ayako Oyane [a,*], Nao Matsuoka [a,b], Kenji Koga [a], Yoshiki Shimizu [a], Maki Nakamura [a], Kenji Kawaguchi [a], Naoto Koshizaki [a,c], Yu Sogo [d], Atsuo Ito [d], Hidero Unuma [b]

[a] Nanomaterials Research Institute, National Institute of Advanced Industrial Science and Technology (AIST), Central 4, 1-1-1 Higashi, Tsukuba, Ibaraki 305-8562, Japan
[b] Graduate School of Science and Engineering, Yamagata University, 4-3-16 Jonan, Yonezawa, Yamagata 992-8510, Japan
[c] Graduate School of Engineering, Hokkaido University, Nishi 8, Kita 13, Kita-ku, Sapporo, Hokkaido 060-8628, Japan
[d] Health Research Institute, National Institute of Advanced Industrial Science and Technology (AIST), Central 6, 1-1-1 Higashi, Tsukuba 305-8566, Japan

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ABSTRACT

Biomimetic calcium phosphate (CaP) precipitation processes using supersaturated CaP solutions are useful in surface functionalization of biomedical materials. We applied our laser-assisted biomimetic (LAB) process to successfully achieve rapid single-step CaP precipitation on the surface of titanium metal, which is an important metallic biomaterial, by applying pulsed laser irradiation to the titanium substrate immersed in a supersaturated CaP solution. Precipitation occurred via the combined effect of laser surface modification and ambient heating. Moreover, we demonstrated immobilization of various contents of osteogenic substances (zinc and fibronectin components) on the titanium surface together with CaP by supplementing the CaP solution with these substances. The LAB process is expected to be a facile and effective surface functionalization technique for titanium-based biomaterials to provide them with osteoconductivity because of CaP and stimulatory effects on bone formation due to osteogenic substances.

Certain types of calcium phosphate (CaP) ceramics such as hydroxyapatite and β-tricalcium phosphate have long been used as orthopedic and dental implants owing to their good biocompatibility and osteoconductivity. However, CaP ceramics are hard and brittle; hence, they are utilizable only under low load-bearing conditions.

Under high load-bearing conditions, implants made of tough and strong metals including titanium metal and its alloys have been used. To provide these metallic implants with osteoconductivity, various CaP deposition techniques such as plasma spraying, sputtering, laser ablation (pulsed laser deposition), and biomimetic processes have been proposed. Among these techniques, biomimetic processes [1–5] utilizing supersaturated CaP solutions as both the CaP source and growth media have recently attracted increased attention. Biomimetic processes are capable of precipitating CaP and concurrently immobilizing biologically functional substances, such as antimicrobials, cytokines, trace elements, and nucleic acids, by taking advantage of pseudo-physiological reaction conditions [6,7].

The weaknesses associated with conventional biomimetic processes are the relatively long processing time required because of the slow CaP precipitation/growth rate in supersaturated CaP solutions and the necessity of subjecting the substrate materials to a prior surface modification step. Examples of surface modification methods required for metallic materials include grit-blasting followed by CaP-precoating [1], NaOH treatment [2], H_2O_2 treatment [3], anodic oxidation [4], and focal laser irradiation [5]. The resulting metals with modified surfaces induce CaP precipitation on their surfaces when subsequently immersed in a supersaturated CaP solution, typically for as long as one day or more.

Recently, we achieved rapid and single-step CaP precipitation on polymer substrates by a laser-assisted biomimetic (LAB) process [8,9]. In this process, pulsed, unfocused laser irradiation was applied to a polymer substrate that was immersed in a metastable supersaturated CaP solution (denoted herein as the CP solution [10]) in accordance with Lee's system [11]. Within 30 min of irradiation, CaP precipitated onto the laser-irradiated polymer substrate surface by the combined effects of laser surface modification and ambient heating at the solid–liquid interface [8,9]. Therefore, the LAB process is expected to be a facile and effective tool for surface functionalization of biomedical materials.

The first aim of the present study was to apply the LAB process for CaP precipitation on the surface of titanium metal. The second aim of this study was to immobilize biologically functional substances onto the titanium surface along with CaP precipitation by the LAB process. The biologically functional substances selected were zinc (Zn), an essential trace element and fibronectin (Fn), a protein facilitating cell adhesion. Both Zn [12,13] and Fn [14] could act as osteogenic substances and are expected to stimulate bone regeneration.

* Corresponding author.
E-mail address: a-oyane@aist.go.jp (A. Oyane).

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To achieve the first aim, the LAB process was carried out on titanium metal substrates using the CP solution, as described in our previous reports [8,9]. Briefly (see Supplementary information for experimental details), a single titanium substrate (1 mm × 10 mm × 10 mm) was immersed in 10 mL of the CP solution maintained at 25 °C using a water bath. Pulsed, unfocused laser irradiation (30 Hz, 355 nm, 4 W/cm^2) was applied to the titanium substrate for 30 min while immersed in the CP solution. The laser-irradiated surface ($\phi = 5$ mm) of the substrate was examined.

In addition to polymer substrates, as reported previously [8,9], the applicability of the CaP precipitation technique using the LAB process was verified for titanium metal substrates as well. As revealed by energy-dispersive X-ray analysis (EDX), Ca and P were newly detected on the titanium surface after conducting the LAB process (Fig. 1a), suggesting the precipitation of CaP compounds. As shown in the scanning electron microscopy (SEM) images in Fig. 1b, the titanium surface deformed into a micro-scale grainy structure with sub-microscale asperity faces after conducting the LAB process. To isolate the morphological effects on the metal surface owing to laser surface modification and CaP precipitation, we examined an equivalently laser-irradiated titanium surface in ultrapure water, which revealed a similar micro-scale grainy structure to that of the surface subjected to the LAB process but without sub-microscale asperity (Fig. S1). Therefore, the microscale and sub-microscale deformations of the substrate are expected to be due to laser surface modification and CaP precipitation, respectively. According to the results of thin-film X-ray diffraction (TF-XRD) shown in Fig. 2a and selected area diffraction by transmission electron microscopy (TEM) shown in Fig. 2b, the CaP precipitates contained hydroxyapatite as a major crystalline phase. The obtained CaP precipitates might also contain the amorphous CaP phase, which was difficult to be identified from the background intensity in Fig. 2(a).

In the LAB process, laser absorption by the titanium substrate should be an essential first step for CaP precipitation, as suggested from our previous results on polymer substrates [8,9]. Absorption of 355 nm light by the titanium substrate was experimentally confirmed by absorption spectroscopy using an ultraviolet–visible–near-infrared (UV–VIS–NIR) spectrophotometer (Fig. S2). The absorbed laser light energy is assumed to be transformed into thermal energy thereby causing not only surface modification of the substrate, but also ambient heating during the LAB process, as detailed in the following paragraph.

As briefly discussed previously, the laser surface modification in the LAB process was elucidated by a control experiment that evaluated an equivalently laser-irradiated titanium surface in ultrapure water. The untreated titanium substrate used in the present study had a naturally-oxidized titanium surface (Fig. S3b). Laser irradiation in ultrapure water caused not only micro-scale deformation (Fig. S1) but also further oxidization of the substrate surface to a rutile phase (Fig. S3). The laser-irradiated titanium surface showed higher hydrophilicity than the untreated surface; the water contact angle on the substrate decreased from 94.8° ± 1.2° to 12.8° ± 1.7° (n = 3) after laser irradiation in ultrapure water. UV light-induced photochemical reaction [15] and/or laser ablation might be involved in these surface reactions, and similar surface reactions are expected to occur in the CP solution as well. The resulting highly-oxidized titanium surface with increased hydrophilicity and roughness is expected to have increased affinity with the CaP components (e.g., Ca, P ions, and CaP nanoclusters [16,17]) in the CP solution, thereby inducing CaP precipitation heterogeneously at the laser-irradiated solid–liquid interface. The above expectation about heterogeneous CaP precipitation is supported by the experimental results that the equivalently laser-irradiated titanium surface in ultrapure water formed a CaP layer on its surface when it was subsequently immersed in the CP solution for 24 h (Fig. S4). On the other hand, when the subsequent immersion period in the CP solution was 30 min (the same as the LAB process), neither Ca nor P was detected by EDX on the laser-irradiated titanium surface in ultrapure water (Fig. S4). This result indicates that the CaP precipitation on the titanium surface by the LAB process is accelerated by the effect of ambient heating due to laser irradiation. To amplify the heating effect, the LAB process was performed on a titanium substrate in the absence of a temperature-controlled water bath. The temperature of the CP solution increased by approximately 9 °C (from 25 to 34 °C) after conducting the LAB process. This increased temperature must be due to the light-to-heat energy conversion at the titanium surface because the CP solution itself exhibits very little light absorption at 355 nm [8], and the temperature rise of the CP solution was negligibly small (<1 °C) in the absence of a titanium substrate. The ambient heating accelerates CaP precipitation by increasing the mass transfer rate and the degree of supersaturation with respect to the CaP compounds [18,19].

Next, we attempted to immobilize the selected biologically functional substances, Zn and Fn, on the titanium surface together with CaP by the LAB process. For this purpose, we supplemented the CP solution with $ZnCl_2$ or Fn at various concentrations. Using the

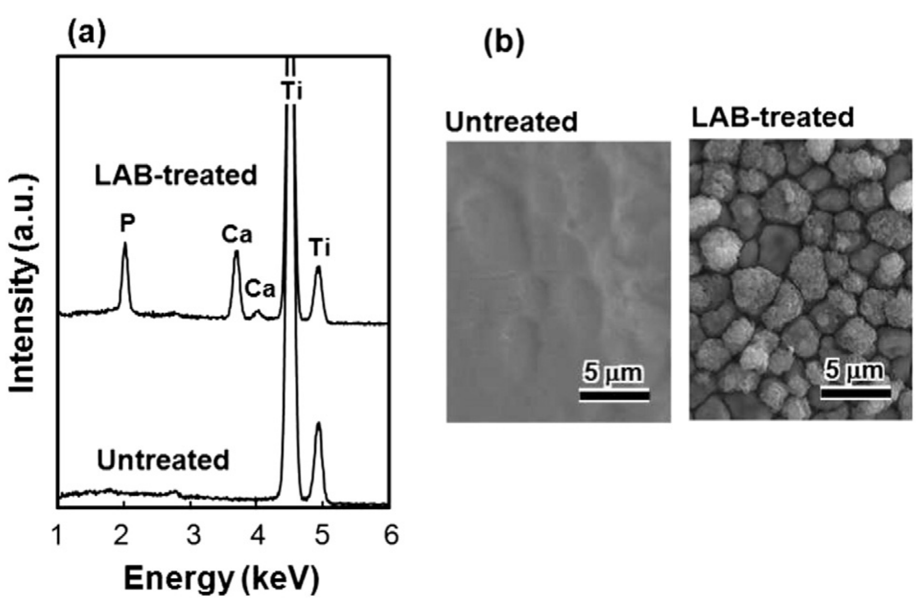

Fig. 1. (a) Energy-dispersive X-ray analysis (EDX) spectra and (b) scanning electron microscopy (SEM) images of the untreated titanium substrate surface and that after conducting the LAB process.