

RSC Advances

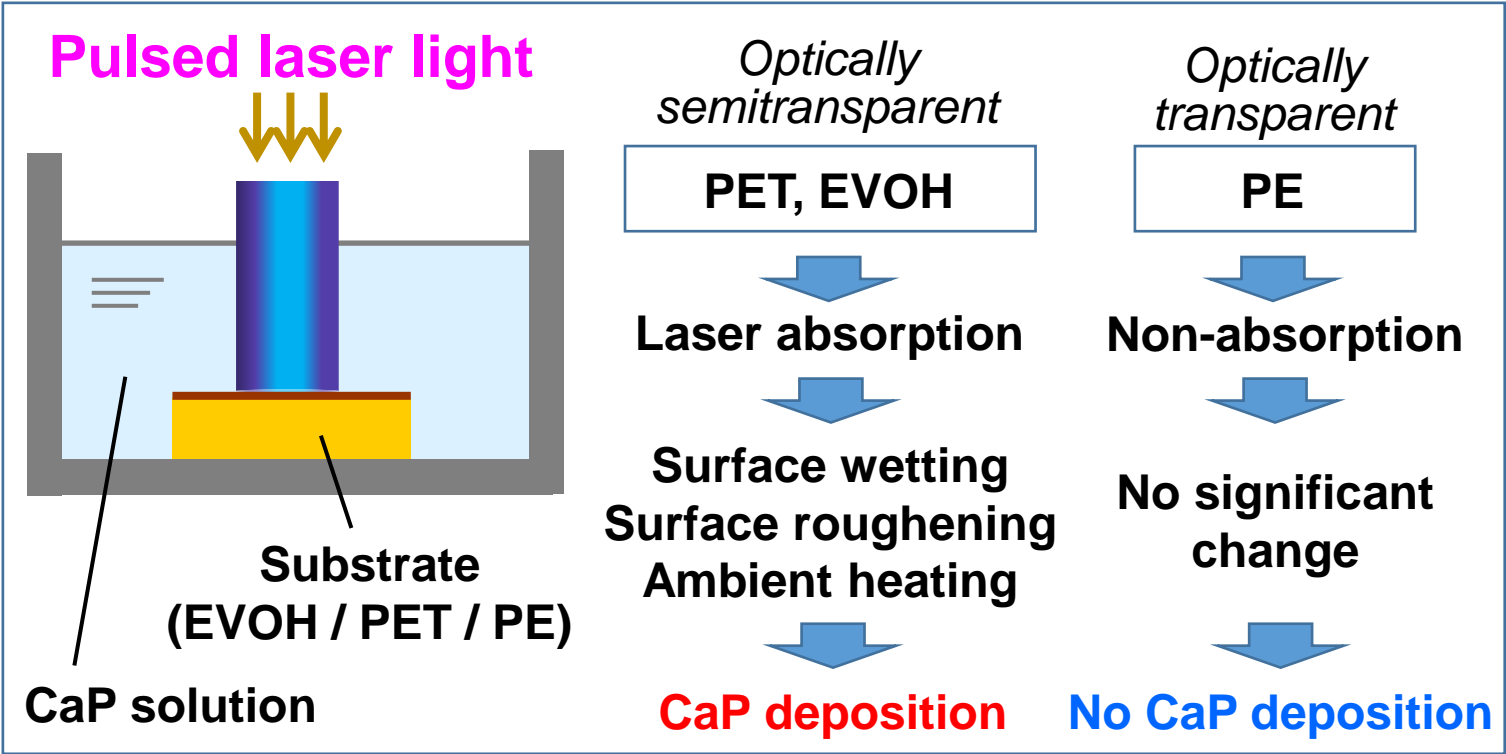


This is an *Accepted Manuscript*, which has been through the Royal Society of Chemistry peer review process and has been accepted for publication.

Accepted Manuscripts are published online shortly after acceptance, before technical editing, formatting and proof reading. Using this free service, authors can make their results available to the community, in citable form, before we publish the edited article. This *Accepted Manuscript* will be replaced by the edited, formatted and paginated article as soon as this is available.

You can find more information about *Accepted Manuscripts* in the [Information for Authors](#).

Please note that technical editing may introduce minor changes to the text and/or graphics, which may alter content. The journal's standard [Terms & Conditions](#) and the [Ethical guidelines](#) still apply. In no event shall the Royal Society of Chemistry be held responsible for any errors or omissions in this *Accepted Manuscript* or any consequences arising from the use of any information it contains.



Calcium phosphate deposition was induced on optically semitransparent polymers irradiated with laser in a supersaturated solution, but not on a transparent polymer.

Cite this: DOI: 10.1039/c0xx00000x

www.rsc.org/xxxxxx

COMMUNICATION

Laser-assisted calcium phosphate deposition on polymer substrates in supersaturated solutions

Ayako Oyane,^{a*} Ikuko Sakamaki,^a Alexander Pyatenko,^a Maki Nakamura,^a Yoshie Ishikawa,^aYoshiki Shimizu,^a Kenji Kawaguchi^a and Naoto Koshizaki^{a,b}⁵ Received (in XXX, XXX) Xth XXXXXXXXX 20XX, Accepted Xth XXXXXXXXX 20XX

DOI:

Calcium phosphate deposition was induced on optically semitransparent polymers, but not on a transparent polymer, by a pulsed laser irradiation process in a supersaturated solution. This process would be useful as a tool for depositing calcium phosphate on laser-absorbing polymers.

Calcium phosphate (CaP) is a major mineral component in vertebrate hard tissues. Thus, some CaP compounds, such as hydroxyapatite and octacalcium phosphate, show good biocompatibility and osteoconductivity¹. The physiological formation of CaP in body fluids can also be reproduced or mimicked in *ex vivo* acellular CaP solutions (typical example is a simulated body fluid) that are supersaturated with respect to CaPs². Such supersaturated CaP solutions are useful as reaction media for inducing *in vitro* biomimetic CaP deposition on the surfaces of biomedical materials^{3,4}. The *in vitro* biomimetic CaP deposition processes using supersaturated CaP solutions offer the advantages of safe and pseudo-physiological reaction conditions compared to conventional physical processes (plasma-sputtering, pulsed laser deposition, ion-beam sputtering, etc.). Such mild reaction conditions enable fine control of composition and structure of CaPs⁵. Furthermore, CaPs can be loaded with a wide range of cell-stimulating substances, such as trace elements, functional proteins, genes, and antigens⁴. Hence biomimetic CaP deposition processes using supersaturated CaP solutions have recently attracted increased attention as tools for the production and surface functionalization of biomedical materials.

Conventional biomimetic CaP deposition processes generally involve two steps: pretreatment of the substrate followed by immersion in a supersaturated CaP solution^{3,6}. In the pretreatment step, a substrate is surface-modified with functional groups or

CaP seeds or both. The surface-modified substrate material is then immersed in a supersaturated CaP solution to grow a continuous CaP layer on the surface. Due to the relatively slow reaction rate, the duration of this immersion step is usually as long as one day or more.

Recently, we developed a laser-assisted biomimetic (LAB) process for CaP deposition on a polymer substrate in which the pretreatment⁶ and immersion steps are combined into a single step⁷. In the LAB process, a substrate of ethylene-vinyl alcohol copolymer (EVOH) is immersed in a supersaturated CaP solution (a so-called CP solution)^{4,7,8} and is irradiated on the surface with a weak Nd-YAG nanosecond pulsed laser (355 nm, 30 Hz, 4–6 W·cm⁻²). Within just 30 min of irradiation, CaP is formed *in situ* on the laser-irradiated region of the substrate. This LAB process is quick and accomplished in a single step without any pretreatment or postimmersion step; hence, it is advantageous over conventional two-step biomimetic processes.

Here, we provide theoretical insight into the LAB process and demonstrate its applicability to polymer substrates other than EVOH. We hypothesize that laser absorption by the substrate should be an essential requirement for inducing CaP deposition at the laser-irradiated surface. To verify this hypothesis, the LAB process was applied to two different polymers: optically transparent polyethylene (PE) and semitransparent polyethylene terephthalate (PET). Substrates of PE, PET, and EVOH as a control were prepared by hot-pressing (ESI†). Absorption spectroscopy confirmed that both the EVOH and PET substrates absorb light energy at 355 nm, whereas the PE substrate hardly absorbs any (Fig. 1).

The LAB process was conducted for each substrate in a CP solution (NaCl 142 mM, K₂HPO₄·3H₂O 1.50 mM, CaCl₂ 3.75 mM, buffered to pH = 7.40 at 25°C)^{4,7,8}. First, the substrate was immersed in 10 mL of the CP solution that was maintained at 25°C via a temperature-controlled water bath. Laser irradiation was performed for 30 min using the output of the third harmonic (355 nm) of a Nd-YAG laser (Quanta-Ray LAB-150-30, Spectra-Physics, USA) operated at a frequency of 30 Hz and an energy density of 2, 4, or 6 W·cm⁻². The laser beam possessed an output diameter of approximately 8 mm and was passed without focusing through a 5-mm-diameter hole of a metallic mask to

^a Nanosystem Research Institute, National Institute of Advanced Industrial Science and Technology (AIST), Central 4,1-1-1 Higashi, Tsukuba, Ibaraki 305-8562, Japan. Fax: +81-29-861-3005; Tel: +81-29-861-4693; E-mail: a-oyane@aist.go.jp

^b Graduate School of Engineering, Hokkaido University, Nishi 8, Kita 13, Kita-ku, Sapporo, Hokkaido 060-8628, Japan

[†] Electronic supplementary information available: Details of substrate preparation and supplementary figures for SEM images, and EDX and FT-IR spectra. See DOI:

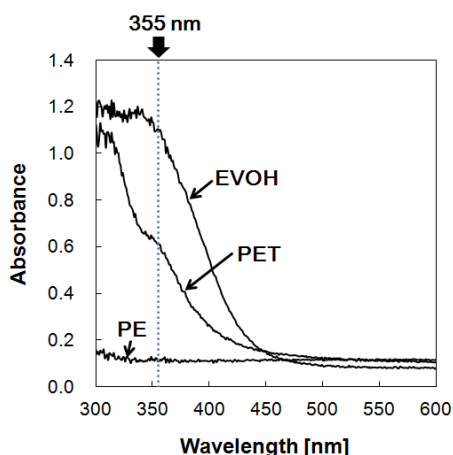


Fig. 1 Absorption spectra of the PE, PET, and EVOH substrates.

irradiate the substrate. After irradiation, the substrate was washed with ultrapure water and then examined for structural changes on the laser-irradiated surface.

After the LAB process, CaP deposited on the PET substrate as reported for the EVOH substrate⁷, but did not form on the PE substrate. On the PE substrate, neither morphological (by scanning electron microscopy (SEM)) nor compositional (by energy-dispersive X-ray spectroscopy (EDX)) changes were found after the LAB process, irrespective of the energy density (Fig. S1†). In contrast to the PE substrate, microscale deformation was observed on the PET substrate after the LAB process (Fig. 2). In the EDX spectra, Ca and P peaks appeared after the LAB process at $4 \text{ W}\cdot\text{cm}^{-2}$, suggesting CaP deposition on the PET surface (Fig. 3a). Similar EDX results were also obtained for the PET substrates after the LAB process at 2 and $6 \text{ W}\cdot\text{cm}^{-2}$. According to electron diffraction analysis by transmission electron microscopy (Fig. 3b and 3c), the deposited CaP included hydroxyapatite. Coexistence of undetectable amounts of other CaP phases, such as amorphous CaP and octacalcium phosphate, cannot be denied.

In the LAB process, laser absorption by the substrate should be prerequisite for CaP deposition. As described above, the LAB process was inapplicable to the optically transparent PE substrate, but applicable to the semitransparent (at 355 nm) PET and EVOH⁷ substrates. It should be noted here that the CP solution has very little laser absorption⁷ and remained apparently clear without inducing homogeneous CaP nucleation even after the LAB process. Thus, the CaP formation on the PET surface was

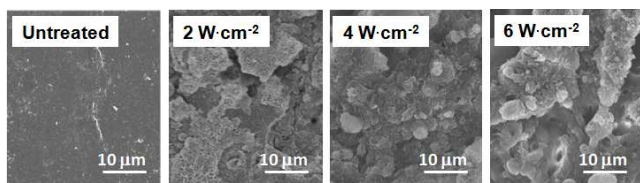


Fig. 2 SEM images of the surface of the PET substrate before and after the LAB process at 2, 4, or $6 \text{ W}\cdot\text{cm}^{-2}$.

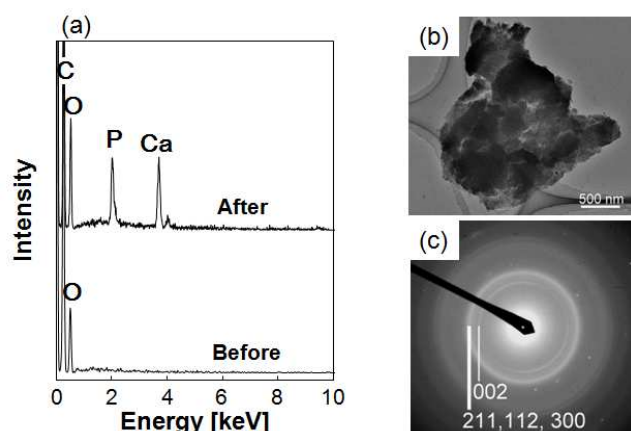


Fig. 3 (a) EDX spectra of the surface of the PET substrate before and after the LAB process at $4 \text{ W}\cdot\text{cm}^{-2}$. (b) TEM image and (c) electron diffraction pattern of the deposit formed on the LAB ($4 \text{ W}\cdot\text{cm}^{-2}$)-treated PET substrate.

not due to sedimentation of homogeneously nucleated CaP particles but due to heterogeneous CaP nucleation at the solid-liquid interface. This CaP nucleation should be triggered by laser irradiation in the CP solution, since CaP deposited only on the laser-irradiated surface of the PET substrate and the non-irradiated surface (outside the irradiated region) of the same substrate was kept unchanged.

The mechanism of CaP deposition on the PET substrate is considered to be similar to that proposed for the EVOH substrate⁷. Briefly, laser absorption by the substrate causes heat generation and surface modifications, thereby providing a favourable environment for CaP deposition at the surface as described below.

Laser-induced heat generation was confirmed by a comparative experiment in which the temperature-controlled water bath was not used in the LAB process. As shown in Fig. 4, for both the PET and EVOH substrates, the temperature of the CP solution increased monotonically from 25°C over time during the LAB process at $4 \text{ W}\cdot\text{cm}^{-2}$. However, such a significant

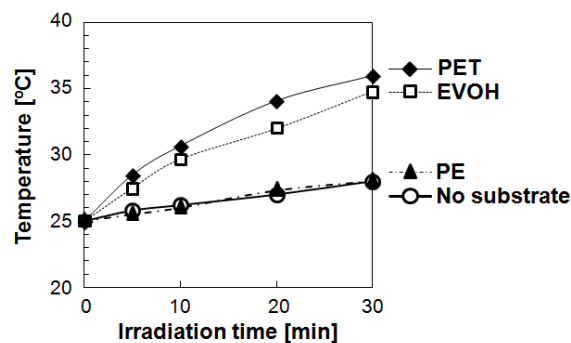


Fig. 4 Changes in temperature of the CP solution during the LAB process at $4 \text{ W}\cdot\text{cm}^{-2}$ for the PE, PET, and EVOH substrates, without a temperature-controlled water bath.

temperature increase was not observed for the PE substrate (the temperature profile corresponded to that without a substrate). Thus, the temperature increase observed for the PET and EVOH substrates was due to laser-substrate interactions. The laser-induced heat generation would accelerate CaP deposition by increasing the mass transfer rate and the degree of supersaturation⁹ of the solution near the substrate surface.

Laser-induced surface modification of the PET substrate was confirmed by a comparative experiment in which laser irradiation (4 W·cm⁻²) was performed in ultrapure water instead of in the CP solution. On increasing the irradiation time from 0 to 10 and 30 min, surface roughness of the PET substrate increased (Fig. 5), and the water contact angle decreased from 92° ± 6° to 65° ± 6° and 49° ± 9° (n = 5), respectively. The increased surface hydrophilicity after irradiation might be caused by cleavage of ester linkages in PET, as revealed by infrared spectroscopy (Fig. S2†)¹⁰. The thus-modified PET surface with ester-derived functional groups (COOH, CHO, OH, etc.) would provide a favourable environment for CaP deposition¹¹. Similar behavior was observed on the EVOH surface that was irradiated in ultrapure water⁷.

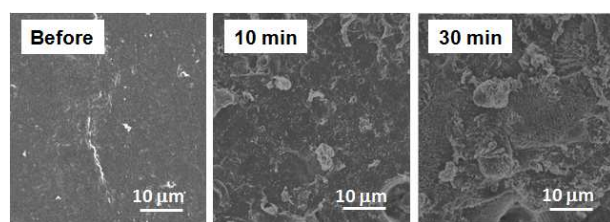


Fig. 5 SEM images of the PET substrate before and after laser irradiation at 4 W·cm⁻² in ultrapure water for 10 min and 30 min.

We consider that the laser-induced surface modifications on the PET and EVOH substrates did not result from thermal deformation or melting. According to the Beer-Lambert law for one-dimensional propagation of light in semitransparent media, we have

$$I = I_0 e^{-kx} \quad (1)$$

where x is the path length, I is the laser-beam energy flow density, and k is an attenuation coefficient that depends on wavelength. The initial laser-beam energy flow density I_0 is obtained from

$$I_0 = \frac{E_0}{S_0 \tau_0} \quad (2)$$

where E_0 is the energy of one individual laser pulse, S_0 is the laser-beam cross section, and τ_0 is the duration of the laser pulse. The attenuation coefficient k can be easily found from the boundary conditions for the inlet and outlet surfaces of the substrate: $E = E_0$ at $x = 0$; $E = E_A$ at $x = \Delta$ (1 mm in our case) by

$$k = \frac{\ln(E_0/E_A)}{\Delta} \quad (3)$$

where E_A is found experimentally in our light absorption data (Fig. 1). Assuming that all the energy absorbed by a hypothetical ultrathin layer with coordinate x is used to heat the part of this layer restricted by the laser-beam cross section (heat transfer is

negligible during the pulse propagation time of 10 ns), we can find the temperature of this layer, T_x :

$$T_x - T_0 = \frac{E_0 e^{-kx} \cdot k}{c_p \cdot \rho \cdot S_0} \quad (4)$$

Here, T_0 is the initial temperature of the substrate (25°C in our case), while c_p and ρ are the heat capacity and density of the substrate material, respectively. Therefore, for the inlet surface of the substrate (*i.e.*, at $x = 0$), we have

$$(T - T_0)_{x=0} = \frac{E_0 \cdot k}{c_p \cdot \rho \cdot S_0} \quad (5)$$

For PET and EVOH, $\rho \approx 1 \text{ g·cm}^{-3} = 10^3 \text{ kg·m}^{-3}$, and $c_p \approx 1\text{--}2 \text{ kJ·kg}^{-1}\text{·K}^{-1}$. Since the interval between laser pulses is long enough (33 ms) to completely cool the substrate, the heating process for one individual laser pulse can be considered. Our numerical estimate shows that the inlet surface temperatures of these polymer substrates increase by just several degrees from their initial temperatures. Nonthermal photochemical reactions¹⁰ might be involved in the laser-induced surface modification in the LAB process, although this should be clarified in a future study.

Not only laser absorption but also the chemistry of the polymers affects the LAB process. In fact, the PET substrate, despite having a smaller light absorption at 355 nm (Fig. 1), showed higher reactivity in the LAB process than the EVOH substrate. According to the SEM (Fig. 2) and EDX results, the PET substrate formed CaP on its surface even by the LAB process at 2 W·cm⁻². However, at this energy level, the EVOH substrate was neither surface-modified nor coated with CaP: it required at least 4 W·cm⁻² for CaP deposition⁷. This might be due to the higher stability of ethylene linkages in EVOH compared to that of the ester linkages in PET.

The LAB process is simple (single-step process), quick, and found to be applicable to several polymers that absorb laser energy. Given these advantages, the LAB process would be useful as a new tool for the production and surface functionalization of biomedical materials made of laser-absorbing polymers. Note that the PET surface was deformed under the tested irradiation conditions and the deposited CaP was not in the form of a dense and continuous layer. Further optimization of the irradiation conditions and *in vitro* and *in vivo* studies should be needed in terms of biomedical applications.

Conclusions

The LAB process for CaP deposition is not specific to EVOH substrates but is potentially applicable to other polymer substrates that absorb laser energy. Laser absorption and the chemistry of the polymer substrate are important factors in the LAB process.

This work was supported by KAKENHI (25108517) from The Ministry of Education, Culture, Sports, Science and Technology (MEXT), Japan.

Notes and references

- M. Jarcho, J.F. Kay, K.I. Gumaer, R.H. Doremus and H.P. Drobeck, *J Bioeng*, 1977, **1**, 79.; H. Aoki, 1991 *Science and medical applications of hydroxyapatite*, Tokyo, Takayama Press System Center.
- P. Koutsoukos, Z. Amjad, M. B. Tomson, and G. H.

- Nancollas, *J Am Chem Soc*, 1980, **102**, 1553.; J.C. Heughebaert, S.J. Zawacki, G.H. Nancollas, *J Colloid Interface Sci*, 1990, **135**, 20.; T. Kokubo, H. Kushitani, S. Sakka, T. Kutsugi and T. Yamamuro, *J Biomed Mater Res*, 1990, **24**, 721.; T. Kokubo and H. Takadama, *Biomater*, 2006, **27**, 2907.
- 3 M. Tanahashi, T. Yao, T. Kokubo, M. Minoda, T. Miyamoto, T. Nakamura and T. Yamamuro, *J Am Ceram Soc*, 1994, **77**, 2805.; A. Oyane, M. Uchida, C. Choong, J. Triffitt, J. Jones and A. Ito, *Biomater*, 2005, **26**, 2407.; A. Oyane, M. Uchida, Y. Yokoyama, C. Choong, J. Triffitt and A. Ito, *J Biomed Mater Res A*, 2005, **75A**, 138.
- 4 A. Oyane, *J Ceram Soc Japan*, 2010, **118**, 77.; X.P. Wang, A. Ito, X. Li, Y. Sogo and A. Oyane, *Biofabrication*, 2011, **3**, 22001.; A. Oyane, X.P. Wang, Y. Sogo, A. Ito and H. Tsurushima, *Acta Biomater*, 2012, **8**, 2034.
- 5 H.M. Kim, K. Kishimoto, F. Miyaji, T. Kokubo, T. Yao, Y. Suetsugu, J. Tanaka and T. Nakamura, *J Biomed Mater Res*, 1999, **46**, 228.; H.M. Kim, K. Kishimoto, F. Miyaji, T. Kokubo, T. Yao, Y. Suetsugu, J. Tanaka and T. Nakamura, *J Mater Sci: Mater Med*, 2000, **11**, 421.
- 6 E. Pecheva, T. Petrov, C. Lungu, P. Montgomery and L. Pramatarova, *Chem Eng J*, 2008, **137**, 144.; B.H. Lee, A. Oyane, H. Tsurushima, Y. Shimizu, T. Sasaki and N. Koshizaki, *ACS Appl Mater Interfaces*, 2009, **1**, 1520.
- 7 A. Oyane, I. Sakamaki, Y. Shimizu, K. Kawaguchi and N. Koshizaki, *J Biomed Mater Res*, 2012, **100A**, 2573.
- 8 M. Uchida, A. Oyane, H. M. Kim, T. Kokubo and A. Ito, *Adv Mater*, 2004, **16**, 1071.
- 30 9 M.R. Christoffersen, J. Christoffersen and W. Kibalczyk, *J Cryst Growth*, 1990, **106**, 349. H. McDowell, T. Gregory and W. Brown, *J Res Natl Bur Stand*, 1972, **81**, 273.
- 10 Z. Zhu and M.J. Kelley, *Polymer*, 2005, **46**, 8883.; M. Dadsetana, H. Mirzadehb and N. Shari, *Rad Phys Chem*, 1999, **56**, 597.
- 35 11 M. Tanahashi and T. Matsuda, *J Biomed Mater Res*, 1997, **34**, 305.; K. Sato, T. Kogure, Y. Kumagai and J. Tanaka, *J Coll Interface Sci*, 2001, **240**, 133.; M. Kawashita, M. Nakao, M. Minoda, H.M. Kim, T. Beppu, T. Miyamoto, T. Kokubo and T. Nakamura, 2003, **24**, 2477.
- 40