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Review

Biological modification of tooth surface by laser-based apatite coating techniques

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ABSTRACT

Background: Development of new clinical regenerative procedures is needed for the reconstruction of the connective tissue attachment lost to periodontal disease. Apatite coating on the affected root surfaces could improve root surface biocompatibility and promote the reestablishment of connective tissue attachment.

Highlight: We developed two novel techniques that use laser light for coating the tooth surface with apatite. In the laser-assisted biomimetic (LAB) process, a tooth substrate was placed in a supersaturated calcium phosphate solution and irradiated for 30 min with low-energy pulsed laser light. Due to the laser-assisted pseudo-biomineralization, a submicron-thick apatite film was created on the laser-irradiated tooth surface. Furthermore, we created a fluoride-incorporated apatite film on the tooth surface using the LAB process and demonstrated its antibacterial activity against *Streptococcus mutans*. In the laser-induced forward transfer with optical stamp (LIFTOP) process, a thin apatite film loaded with the cell-adhesion protein, fibronectin, was prepared beforehand as a raw material on the optical stamp (carbon- and polydimethylsiloxane-coated support) by a conventional biomimetic process. After irradiation with a single laser pulse, the film (microchip) was transferred onto a tooth substrate via laser ablation of the carbon sacrificial layer. The LIFTOP process requires only a short processing time and has a minimal heat effect on the film; thus, the film exhibits cell adhesion activity even after the LIFTOP process.

Conclusion: The LAB and LIFTOP processes have the potential as novel tools for tooth surface modification in the treatment of periodontal disease.

Contents

Abbreviations: CaP, calcium phosphate; Fn, fibronectin; LAB, laser-assisted biomimetic; LIFTOP, laser-induced forward transfer with optical stamp; ns, nanosecond; PDMS, polydimethylsiloxane; PET, polyethylene terephthalate.

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1. Introduction

Periodontitis is an infectious disease caused by the formation of oral bacterial biofilms on tooth surfaces. Inflammatory responses to periodontal disease cause the destruction of the connective tissue that forms an attachment between the cementum and gingiva [1]. The connective tissue attachment plays an important role in the function of eating. In the treatment of chronic periodontitis, scaling and root planing are performed to remove biofilm and calculus from the diseased tooth root surface. However, in many cases, regeneration of connective tissue attachment cannot be achieved on the treated tooth surface due to the epithelial downgrowth along the damaged cementum surface [2].

Various methods have been developed to regenerate the attachment apparatus. Guided tissue regeneration is a conventional method to inhibit epithelial downgrowth and induce periodontal regeneration by placing a membrane at the cervical region of the tooth [3]. Application of signaling molecules, such as enamel matrix derivatives or basic fibroblast growth factor, is another conventional method to promote periodontal regeneration on the treated tooth surface [3,4]. However, such strategies remain insufficient for complete reconstruction of the attachment to the treated tooth, especially for severe cases of periodontal tissue destruction. Therefore, a more reliable treatment technique is needed to reconstruct the attachment.

Our strategy was to promote connective tissue attachment to the treated tooth by improving the tooth surface biocompatibility through apatite coating. Apatite, a calcium phosphate compound abundantly present in teeth, exhibits good compatibility with both soft and hard tissues. Synthetic apatite has been used clinically not only as a bone grafting material but also as a coating material for dental implant fixtures [5]. A variety of apatite coating techniques have been proposed, including plasma spraying, sputtering, powder spraying, pulsed laser deposition, and biomimetic processes [5]. Although some of these coating techniques have been employed for tooth surface modification, none are clinically approved, either for practical or safety reasons. This review describes two laser techniques that we have developed recently for modifying tooth surfaces with a thin film of apatite.

2. Modification of tooth surface by a laser-assisted biomimetic (LAB) process

A laser-assisted biomimetic (LAB) process was developed as a facile and area-specific apatite coating technique on various substrates [6,7]. Since the LAB process is simple (one step) and quick (within 30 min), and allows area-specific apatite coating, this approach would be a suitable method for tooth surface modification in the clinical setting. Specifically, we attempted modification of the tooth surface with apatite using the LAB process. The LAB process was performed by immersing a human tooth substrate in a supersaturated calcium phosphate (CaP) solution and then irradiating the surface with low-energy pulsed laser light for a few tens of minutes (Fig. 1, left). Surface analysis demonstrated that a submicrostructured apatite film was formed on the tooth surface following the LAB process (Fig. 1, right) [8]. The formation of this apatite film resulted from pseudo-biomineralization induced at the laser-irradiated tooth surface via laser surface etching and heating effects. Cross-sectional analysis revealed that the tooth substrate was coated with a submicron-thick film that was composed of pillar-like apatite nanocrystals with a weak c-axis orientation perpendicular to the

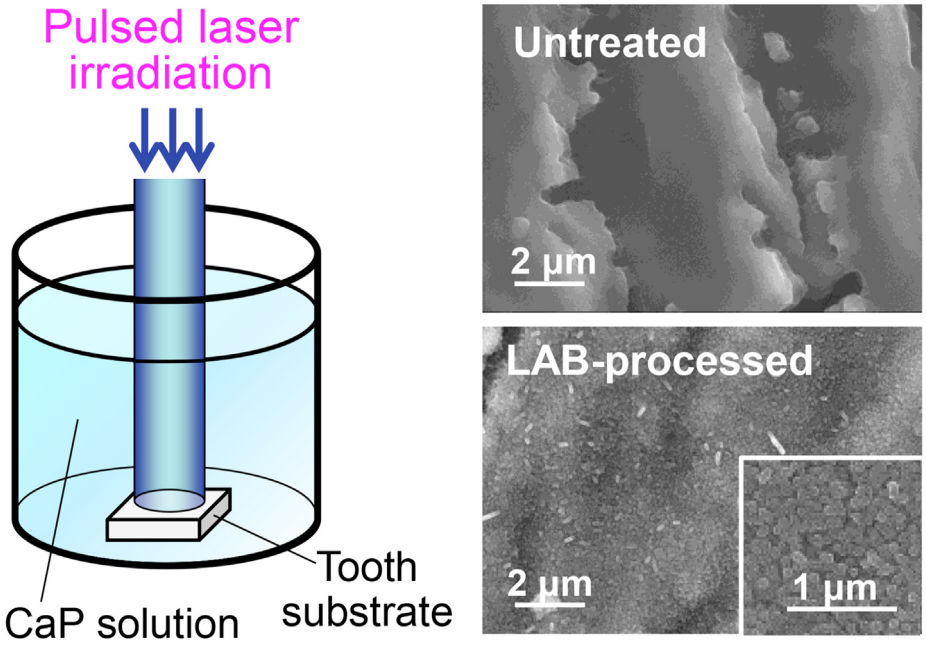

Fig. 1. Schematic of the LAB process (left) and scanning electron microscope images of the surfaces of the tooth substrate before (upper right) and after (lower right) the LAB process using the CaP solution [8]. CaP, calcium phosphate; LAB, laser-assisted biomimetic.

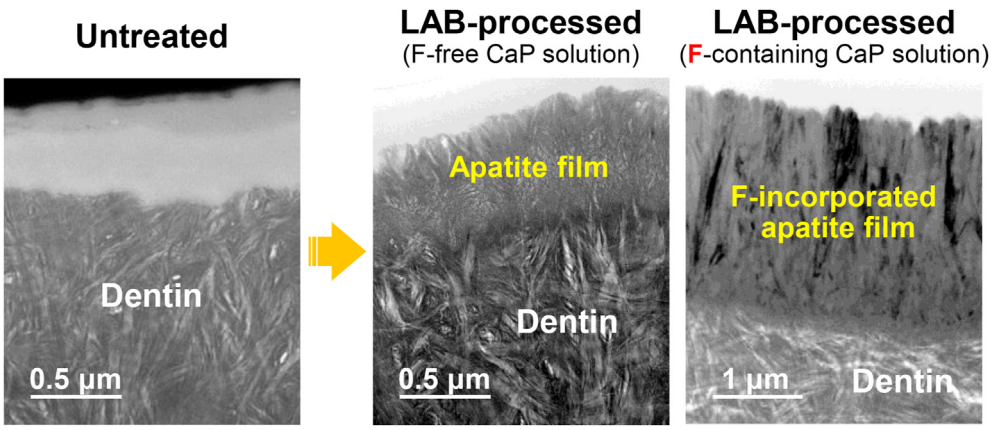

Fig. 2. Cross-sectional transmission electron microscope images of the tooth substrates before (left) and after (center, right) the LAB process using the CaP solution with (right) and without (center) fluoride ions [8,12]. CaP, calcium phosphate; LAB, laser-assisted biomimetic.

tooth surface (Fig. 2, center). This thin film was seamlessly integrated with the underlying tooth substrate without any apparent gaps. Previously, we demonstrated that a LAB-processed polymer substrate coated with apatite exhibited improved cyto-compatibility compared to the untreated substrate [9]. Hence, we speculate that the LAB-processed tooth surface would show improved cyto-compatibility compared to the infected or conventionally treated tooth root surface.

In the LAB process, a specific element can be incorporated into the newly formed apatite film by adding that element to the CaP solution [10,11]. For instance, the authors applied the LAB process to a tooth substrate using a fluoride-containing CaP solution. As a result, a thin film of fluoride-incorporated apatite was formed on the LAB-processed tooth substrate (Fig. 2, right) [12]. The LAB-processed tooth substrate showed antibacterial activity against *Streptococcus mutans*, a major bacterium in the oral cavity, most likely due to fluoride ion release from the surface film. Reportedly, fluoride ions possess antibacterial activity against various oral bacteria [13]. Therefore, the LAB-processed tooth surface with fluoride-incorporated apatite coating is expected to prevent regrowth of bacteria and to promote healing of periodontal tissues in vivo.

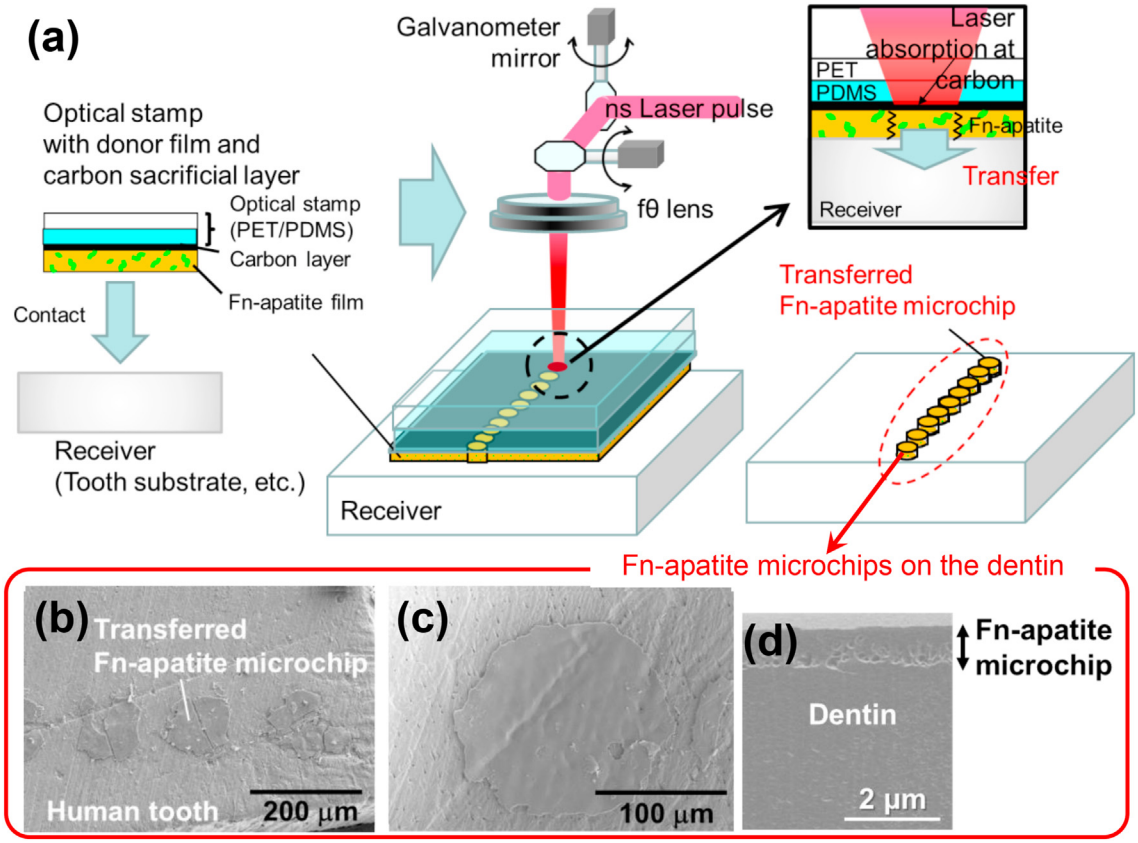

Fig. 3. (a) Schematic diagram of the LIFTOP process and (b–d) scanning electron microscope images of Fn-immobilized apatite (Fn-apatite) transferred to the tooth substrate. (b, c) Surface observation images and (d) cross-sectional image [14]. Fn, fibronectin; LIFTOP, laser-induced forward transfer with optical stamp; ns, nanosecond; PDMS, polydimethylsiloxane; PET, polyethylene terephthalate.

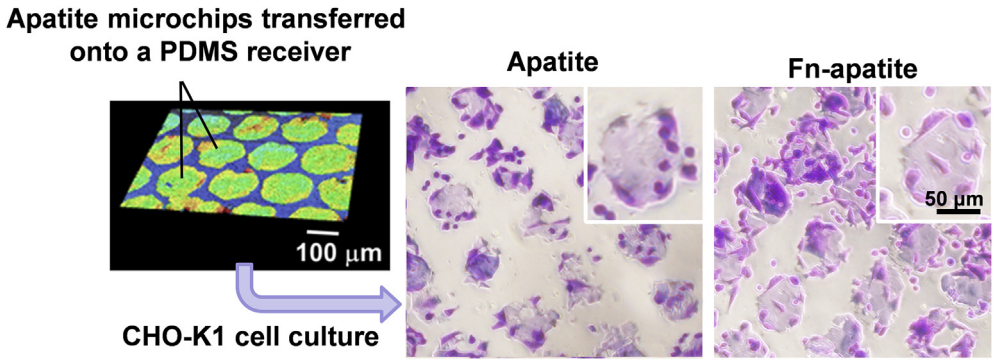

Fig. 4. Cell affinity test results for the apatite without Fn and Fn-apatite microchips transferred on a PDMS substrate [15]. Crystal violet staining (right panels). Fn, fibronectin; PDMS, polydimethylsiloxane.

3. Modification of tooth surface by the laser-induced forward transfer with optical stamp (LIFTOP) process

Currently, the LAB process requires at least 5 min to permit the formation of fluoride-incorporated apatite on the tooth surface [12]. To further speed up the coating process, we employed the Laser-Induced Forward Transfer with OPtical stamp (LIFTOP) process for tooth surface modification. The LIFTOP process originally was developed to permit the transfer of materials from a shock-absorbing optical stamp to a target substrate using laser-induced process, such as ablation, of a sacrificial layer as a driving force [14]. As an optical stamp, we used a transparent polyethylene terephthalate (PET) substrate coated with a polydimethylsiloxane (PDMS) shock-absorbing layer. In this work, a carbon sacrificial layer was deposited on the PDMS shock-absorbing layer; this sacrificial layer effectively absorbed the laser pulse in place of the apatite without absorbing light at the laser wavelength. Prior to the LIFTOP process, a thin apatite film was prepared as a raw material on the optical stamp by a conventional biomimetic process (immersion in a CaP solution without laser irradiation). Then, the LIFTOP process was performed for the tooth substrate by irradiating the apatite film/carbon sacrificial layer/PDMS/PET (from the PET side) with a single laser pulse (Fig. 3a).

As a result of the LIFTOP process, an apatite microchip with a shape corresponding to the laser beam spot was transferred onto the tooth substrate (Fig. 3b and c) [14]. The processing time was fairly short; for example, about 80% of a 1-mm-square region was coated with apatite within about 0.1 s using a laser beam with a diameter of 100 μm, and a pulse repetition rate of 1 kHz. Cross-sectional observations showed that the transferred apatite microchip attached to the tooth surface without noticeable gaps (Fig. 3d). The integration at the tooth–apatite interface depends on the laser irradiation conditions and the properties of the apatite film preformed on the optical stamp. Further optimization of the laser irradiation conditions and the preformed apatite films will be needed in future experiments.

This process also is effective for use with multifunctional apatite coatings incorporating biomolecules, such as cell adhesion proteins and growth factors given that laser light is absorbed not by the apatite film but by the undercoated carbon sacrificial layer on the optical stamp. The authors prepared an apatite film loaded with the cell adhesion protein fibronectin (referred to as Fn-apatite) on the optical stamp with the carbon sacrificial layer, and transferred the Fn-apatite to the tooth surface by the LIFTOP process (Fig. 3) [14]. This approach may be of particular value in tooth surface modification, if the LIFTOP process does not cause complete deactivation of biomolecules incorporated in the apatite film. As a demonstration of this strategy, we conducted a cell adhesion test of the Fn-apatite source film and Fn-apatite microchips transferred onto a PDMS receiver substrate [15]. In this test, we fabricated the apatite (without fibronectin) and Fn-apatite films on a carbon sacrificial layer/PET substrate instead of using the optical stamp. The results suggested that the cell adhesion activity of the Fn-apatite film was retained (to some extent) even after film transfer by laser irradiation [15]. As shown in Fig. 4, CHO-K1 cells adhered to, and spread well on, the Fn-apatite microchips with greater avidity than on the apatite microchips (lacking fibronectin), suggesting the biological effect of fibronectin.

4. Conclusion

We have shown that the tooth surface can be rapidly coated with apatite by either of two laser techniques, the LAB and LIFTOP processes. Biofunctional substances (fluoride and fibronectin) incorporated in the thin apatite film exhibited biological effects (antibacterial activity and cell adhesion activity, respectively). These techniques have potential for use in treatment of periodontitis, and could serve as new tools for tooth surface modification. These techniques also potentially could be applied to the treatment of dental caries, given that demineralization of the tooth surface is involved in the caries formation. These techniques deserve further study as novel tools for use in a new era of laser dentistry.

Ethics approval

Human teeth were obtained from a patient attending Hokkaido University Hospital. Informed consent was obtained prior to the collection of human teeth. The protocol for the clinical study was reviewed and approved by the Hokkaido University Hospital Institutional Review Board for Clinical Research (Approval Nos. 17-222 and 20-352).

CRediT authorship contribution statement

Hirofumi Miyaji: Conceptualization, Funding acquisition, Investigation, Methodology, Project administration, Supervision, Visualization, Writing – original draft. **Ayako Oyane:** Conceptualization, Funding acquisition, Investigation, Methodology, Project administration, Supervision, Visualization, Writing – review & editing. **Aiko Narazaki:** Conceptualization, Funding acquisition, Investigation, Methodology, Project administration, Supervision, Visualization, Writing – review & editing.

Conflicts of interest

The authors have no conflict of interest to declare.

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