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Bio-Hybrid: The Next Gen Implant

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Abstract: Osseo-integrated dental implants have been widely used for the rehabilitation of tooth loss. Although dental implants are considered an available treatment in the paradigm shift from traditional dental therapies, such as fixed dental bridges and removable dentures, the fundamental problems must be overcome prior to their clinical use in young patients who are still undergoing jawbone growth. A bio-engineered functional bio-hybrid implant that is combined with adult-derived periodontal tissue and attached with bone tissue can act as a substitute for cementum. This bio-hybrid implant was successfully engrafted and it restored physiological function, including bone remodelling, regeneration and appropriate responsiveness to noxious stimuli. Thus, this article reviews the functional bio-hybrid implant's potential for clinical use as a next-generation dental implant using adult-derived tissues.

Keywords: Bio-hybrid, Cementum, Implant, Osseo Integration, Periodontal ligament.

I. INTRODUCTION

The oral functions associated with mastication and enunciation are important aspects of good health and quality of life[1]. Teeth & its associated periodontal tissue play important roles in both occlusal function and sensory function[1, 2]. Dental disorders such as caries, periodontal disease and traumatic injury cause fundamental oral and general health problems because tooth and periodontal tissue establish the functional cooperation with the maxillofacial region[1].

To restore the occlusal function after tooth loss, conventional dental treatments that replace the tooth with artificial materials, such as fixed dental bridges or removable dentures, have been widely performed[5, 6]. Recently, Osseo-integrated dental implants that are able to stand alone in the jawbone without invading the adjacent teeth have been used to rehabilitate tooth loss[7, 8]. However, because of the absence of natural periodontal tissue, the currently used dental implants that are directly connected to the surrounding alveolar bone do not provide the same function as the physiological tooth, such as the alleviation of excessive occlusal force, orthodontic movement via bone remodelling and the ability to perceive noxious stimuli[4, 7, 8]. It is, therefore, necessary to develop a functional dental implant that cooperates with the maxillofacial region and satisfies physiological functions through a biological connection with a bioengineered PDL.

Current advances in future regenerative therapies have been influenced by many previous studies, including those in the fields of embryonic development, stem cell biology and tissue engineering technologies[9—11]. Stem cell transplantation, which targets structural and functional diseases, has been attempted to repair damaged tissues[12—15]. These stem cells are thought to be a potential resource for stem cell-mediated tissue repair, including dentin or pulp regeneration, based on their high proliferation and multi-differentiation capacity[19]. Periodontal ligament-derived stem cells (PDLSCs), which can differentiate into all periodontal cell types after transplantation, have also been identified[19, 20].

To restore tooth loss through the use of dental implants and stem cells, some studies reported periodontal tissue formed on implants using periodontal ligament stem cells[24, 25]. However, PDL structure, which was equivalent to a natural tooth, and physiological functions were not demonstrated. Then, a novel fibrous connected implant that uses embryonic dental follicle stem cells has been successfully demonstrated as a proof-of-concept for a next-generation bio-hybrid dental implant[26].

This technique focused on the substitution of alveolar bone for cementum because the structural and biological properties of cementum are almost similar to those of alveolar bone. The technique aimed to develop a novel bioengineering method consisting of adult-derived PDL tissue and a dental implant attached to bone tissue as a substitute for the cementum.

II. MATERIALS AND METHODS ANIMALS

Tissue Isolation of Rat Periodontal ligament (PDL) Tissue

1. Tooth germs were extirpated at embryonic day (ED) 14.5, 18.5 and at postnatal day (PD) 7, and mature teeth were extracted at (PD) 35.
2. The isolated tooth germs were incubated in 50 U/ml dispase (BD, Franklin Lakes, NJ, USA) for 2 min at room temperature as a brief enzymatic treatment for the separation of the dental mesenchyme, or dental follicle tissue, from the tooth germ.
3. After enzymatic treatment, the mesenchymal tissue and dental follicle tissues (DF) were separated using a fine needle in Dulbecco's modified Eagle's medium (D-MEM; Kohjin bio, Saitama, Japan) supplemented with 10% foetal calf serum (GIBCO, Grand Island, NY, USA), 100 U/mL penicillin (Sigma, St. Louis, MO, USA), 100 mg/mL streptomycin (Sigma) and 70 U/mL Deoxyribonuclease I from bovine pancreas (DNase I; Sigma).
4. The periodontal ligament tissues were seceded from PD35 in the first molar using a surgical knife.
5. Each of the isolated tissues was wrapped around hydroxyapatite particles (HA, CALCITITE; Hakuho, Tokyo, Japan) of approximately 50 mm in diameter.
6. These HA particles were placed onto a cell culture insert (0.4 mm pore diameter; BD) and incubated at 37uC for 24 hours, and they were transplanted into a subrenal capsule for 30 days using syngeneic C57BL/6 mice (8 week-old, female) as the host.

Implant Fabrication and Surface Analysis

1. The implants were made of pure titanium wire (Nilaco, Tokyo, Japan) with a length of 1.7 mm and a diameter of 0.6 mm, and their apical sides were shaved into a conical shape.
2. To promote cementum deposition around the implants, their surfaces were coated with HA, which is biocompatible and osteoinductive, via sputter deposition[27] (1–2 mm thickness of HA; Yamahachi dental MFG., Co., Aichi, Japan).
3. DF tissues from ED18.5 mice were wrapped around the HA implants (5–6 tissues of each).
4. The titanium and HA implants were coated with platinum, and their surfaces were observed using an S- 4700 (Hitachi High-Tech, Tokyo, Japan) scanning electron microscope operated at 5 kV.

Transplantation of Implants

1. The lower first molars of 4-week-old C57BL/6 mice were extracted under deep anesthesia, and the resulting bone wounds were allowed to heal for 2–3 weeks.
2. An incision of approximately 2.0 mm in length was made through the oral mucosa at the extraction site with a surgical knife to access the alveolar bone.
3. A dental drill (NSK, Tochigi, Japan) and root canal files (MANI, Tochigi, Japan) were used to create a bony hole of approximately 0.8 mm in diameter and 1.3–1.5 mm in depth in the exposed alveolar bone surface.
4. The HA and bio- hybrid implants were transplanted into the bony hole, and the incised oral mucosa was sutured with 8-0 nylon (Bear Medic, Chiba, Japan).
5. To generate a critical size bone defect model (3-wall bone defect model) which could not heal spontaneously, the buccal supporting alveolar bone (0.7 mm in mesiodistal width and 1.5 mm in depth) in the lower first molar extracted region was removed using a dental drill under deep an aesthesia.
6. The implants were transplanted into these defects using the same procedure described above.

Experimental Orthodontic Treatment

1. Experimental tooth movement was achieved with a horizontal orthodontic force of approximately 10–15 g that was applied continuously to the bio-hybrid implants of mice in the experimental group in the buccal direction using a dial tension gauge (Mitsutoyo, Kanagawa, Japan) for 3, 7 or 14 days.

2. In the control group, the orthodontic force was applied in the buccal direction to the first molars of 7-week-old normal C57BL/6 mice in the same manner as the experimental group.
3. Serial sections from day 6 samples were analysed by in situ hybridisation analysis for macrophage colony-stimulating factor-1 (Csf-1) and osteocalcin (Ocn) mRNA, as previously described[27, 28].
4. The orthodontic movement distance of the bio- hybrid implants and natural first molars was measured using TRI/3D-BON software (Ratoc, Osaka, Japan).

III. RESULTS

Fabrication of the Bone-attached Implant

The bone-attached implant was enveloped with isolated adult-derived PDL tissues and transplanted into the first molar region in the mandible of an immunodeficient mouse (Fig A). To produce a bio-hybrid implant with bioengineered periodontal tissue, it is necessary to use the alveolar bone formation on the implant surface as a substitute for cementum. Researchers investigated whether a bone-attached implant could be generated by using the current osseo-integrated implant as an alternative tissue for cementum. To adhere the alveolar bone to the implant, hydroxyapatite (HA) sputtering was used to create a ragged structure on the titanium implant surface, and the implants were transplanted into the lower first molar region of mice in a murine tooth-loss model (Fig. 1B). The HA-coated implant was osseo-integrated in the lower jawbone at 30 days after transplantation (Fig. 1C), and micro-CT and histological analysis revealed proper bone formation and bone attachment around the implant (Fig. 1D, E). After the osseo-integration of the HA-coated implant, researchers isolated a bone-attached implant with a thin layer of bone tissue (approximately 500 µm thickness) around the implant surface as a result of the surgical procedure (Fig. 1F).

Transplantation of a Bio-hybrid Implant into a Tooth-loss Region

PDL tissues were isolated from extracted incisors in the rat by surgical procedure. These tissues were observed a typical fibrous tissue structure (Fig. 2A, B). Researchers next evaluated whether a bone-attached implant combined with adult-derived PDL tissues could serve as functional implant-formed periodontal tissue after engraftment in a tooth-loss region in the adult murine oral environment. Micro-CT images revealed that the periodontal ligament space was observed around the bone-attached implant at 40 days after transplantation (Fig. 2C). Samples that have periodontal ligament space on more than 50% area of the bone-attached implant from CT images could be seen in frequencies of 23/42. In others sample, implants fell out of mandibular or got the osseo-integration. Fallen out samples could have been primarily caused by inflammation of graft rejection. Histological analyses demonstrated that the correct periodontal tissue structure was observed on the bone-attached implant at 40 days after transplantation (Fig. 2D). The PDL fibre structure of the engrafted bone-attached implant, which consisted of transverse collagen fibres, was equivalent to that of a natural molar tooth.

The Bio-hybrid Implant's Response to Mechanical Stress

It has been considered that a functional implant could be achieved by fulfilling physiological tooth functions in the oral environment, such as cooperation with the oral and maxillofacial regions through the PDL. When researchers evaluated orthodontic movement using mechanical force in an experimental tooth movement model, the engrafted bio-hybrid implant moved in a manner similar to that of natural teeth in response to orthodontic force. During an experimental tooth movement model, colony-stimulating factor-1 (Csf-1) mRNA-positive and osteocalcin (OCN) mRNA-positive osteoblasts were observed individually on the compression and tension sides as a result of gene expression patterns (Fig. 3).

The Bio-hybrid Implant's Potential for Perceiving Noxious Stimuli

Teeth are a peripheral target organ in the maxillofacial region for the perception of the trigeminal and sympathetic nerves, which play essential roles in homeostasis and protection. The potential for perceiving noxious stimuli, including occlusal force and pain, is important for proper tooth function. Trigeminal neurons, which innervate the pulp and PDL, can respond to these stimuli and transduce the perceptions to the central nervous system. Immunohistochemical analysis was used to investigate whether an engrafted bio-hybrid implant had neural functions. Anti-neurofilament (NF)-immunoreactive nerve fibres were detected in the PDL of the bio-hybrid implant (Fig. 4A). c-Fos immunoreactivity is induced in the superficial layers of the medullary dorsal horn by noxious stimuli; e.g. electrical, mechanical and chemical stimulation of intraoral receptive fields involving the tooth pulp and the peripheral nerves of the PDL. In the engrafted bio-hybrid implant, c-Fos immunoreactive neuron expression was detectable as much as a natural tooth in the superficial layers of the medullary dorsal horn following noxious stimulation 2 hours after orthodontic treatment (Fig. 4B).

IV. DISCUSSION

The technique has demonstrated the successful engraftment of a functional dental implant via the biological fibrous connectivity system using a bone-attached implant and adult-derived periodontal ligament tissues. It has also shown the subsequent restoration of physiological tooth functions, such as the response to mechanical stress and the potential for perceiving noxious stimuli.

Cementum is a characteristic hard tissue covering the surface of the tooth root. It contributes to essential tooth functions, including protecting the dentin and ensuring a tight connection between the tooth and alveolar bone via the insertion of PDL fibres[2, 29]. Periodontal tissues, which are composed of cementum, the periodontal ligament, and alveolar bone, arise from dental follicle tissue that is derived from the dental mesenchyme in the developing embryo[29]. After tooth development, immature cementoblast cells are thought to be maintained by a self-repair system in cases of the tooth and periodontal tissue injuries[29], [30].

Based on the understanding of cementum development, many previous studies have attempted to reconstruct bioengineered periodontal tissue via cementum formation onto tooth root or dental implant surfaces using scaffolds and dental stem cells[24, 25, 31, 32]. However, no bioengineering method has yet been able to create functional cementum[29].

It is well known that the structural and biological properties of cementum, such as the component ratio of organic (e.g., collagens) to inorganic (e.g., hydroxyappatite) matters and the gene expression patterns in calcified tissue formation, are similar to those of alveolar bone[29, 33]. It is also thought that alveolar bone might be substituted for cementum because cementum has a potential for a physiological reaction to mechanical stress and because alveolar bone exhibits remodelling in the case of orthodontic treatment[34, 35]. In this study, we developed the functional implant with adult-derived PDL tissue and the therapeutic dental implant attached to bone tissue as a substitute for cementum. The engrafted functional implant showed the proper insertion of PDL fibres as a result of bone remodelling. These findings suggest that the bioengineering method used for our functional implant has potential as an available dental implant treatment in the future.

The PDL, which can cooperate with the maxillofacial region through the fibrous connection with the cementum and the alveolar bone, plays important roles in biological tooth functions, including the absorption of occlusal force and tooth movement via bone remodelling[2, 4]. Many studies have attempted to restore the periodontal tissue structure on an implant surface, e.g., using material-based approaches that were incorporated into the subsidence mechanism against occlusal force[36] and biochemical approaches coated by inducible factors[37, 38]. However, these technologies could not completely replace the correct structure and restore the proper functions of periodontal tissue[4].

In this recent technique, researchers have developed a bio-hybrid implant that restored physiological functions through the proper PDL connection. However, critical issues with suitable adult cell sources and bioengineering technology must be resolved for the bio-hybrid dental implant[26]. In the current study, researchers demonstrated the successful engraftment of a functional bone-attached implant with adult-derived PDL tissues that could replicate both the correct periodontal structure on the implant surface and the PDL's function of responsiveness to mechanical stress through bone remodelling. These findings indicate the fulfilment of a therapeutic concept via a next-generation bio-hybrid implant that can achieve functional cooperation by reconstructing fibrous connective tissue, such as the PDL.

Teeth are a peripheral target organ for the sensory trigeminal and sympathetic nerves[39, 40], and the proper function of the nervous system in the maxillofacial region plays essential roles in the regulation of tooth physiological functions and the perception of external stimuli, such as pain and mechanical stress[1, 39]. These physiological functions regarding oral and general health are achieved by the functional cooperation of the teeth, masticatory muscles and temporo-mandibular joint under the control of the central nervous system[39]. It is thus thought that the recovery of the nervous system, which is associated with the re-entry of nerve fibres following the transplantation of a tooth germ or autologous tooth, is critical to fully physiological dental therapy for tooth loss[28, 41].

Current dental implants that are directly connected to the alveolar bone are not able to sense noxious stimuli because of the absence of nerve innervation in periodontal tissue[7, 40]; therefore, it is anticipated that a next-generation bio-hybrid implant will realise the functional recovery of the neuronal perceptive potential for noxious stimuli[4, 26, 40]. This technique has demonstrated that the functional implant was successfully innervated with the peripheral nerves via bioengineered periodontal tissues, and the responsiveness to orthodontic stimulation was restored. These findings indicate that bio-hybrid implants offer the potential for the recovery of neuronal function via proper innervation.

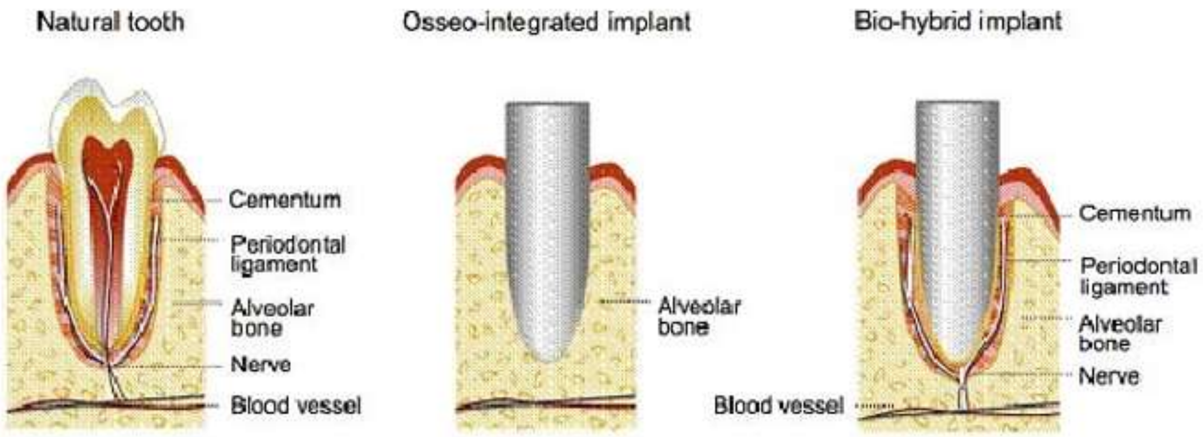
This bioengineering technique might be early applicable to the clinical site because there is no time for cell culture. However, to establish novel treatment with bio-hybrid implants, identification of tissue/ cell source should be needed. Previous studies used stem cells derived from third molar tooth or tooth germ tissue, including of the dental pulp, periodontal ligament, and dental follicle, to repair damaged tissues[19]. The PDL tissues derived from the third molar tooth might be a potential source for treatment with the bio-hybrid implant.

Further studies that optimise bioengineering methods in combination with current dental implant procedures and available stem cells and apply practical analyses in a large animal model will be required before biological dental regenerative therapy can be used clinically.

V. CONCLUSION

In conclusion, this study shows that the transplantation of a bone-attached implant with adult-derived PDL tissues can achieve the formation of proper periodontal tissue-like structures similar to those of the natural tooth, and the engrafted implant has physiological PDL functions that can cooperate with surrounding tissues. This study represents a substantial advance in the development of bio-hybrid implants as a next-generation dental implant therapy using an adult-derived tissue. This study indicates the potential for dental regenerative therapy using a novel bioengineering method and the application of current dental implant treatments.

Figures



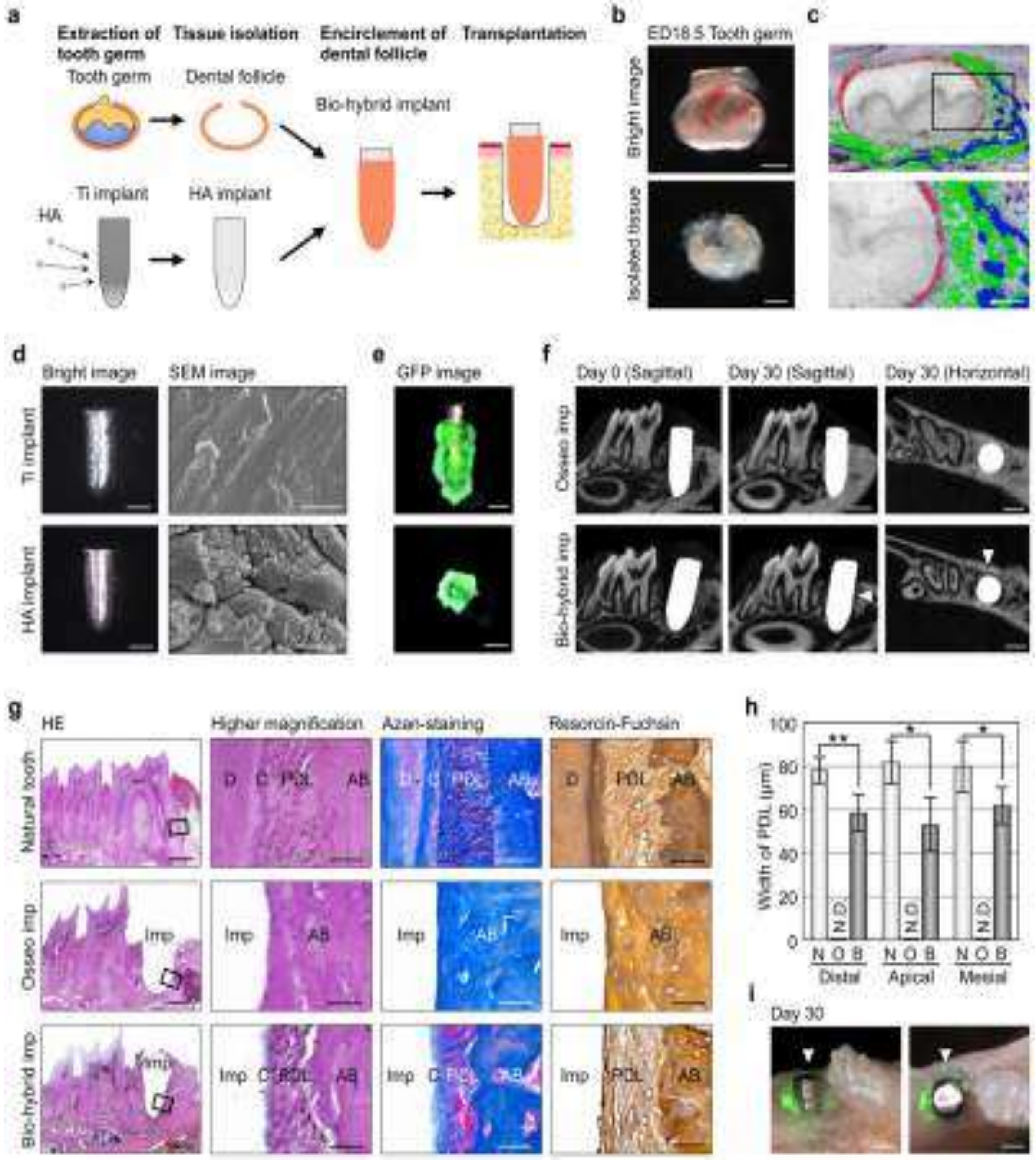


FIG. 1. Fabrication of the Bone-attached Implant

(A) Schematic representation of the development and transplantation technology of the functional implant.
(B) Photographs (upper) and surface analysis (lower) of an HA implant using SEM. Scale bar, 500 µm and 2.0 µm in the photographs and SEM images, respectively.
(C) Photograph of an engrafted osseo-integrated implant. Scale bar, 500 µm.
(D) Micro-CT image of an engrafted osseo-integrated implant. An image represents the sagittal section. Scale bar, 500 µm.
(E) HE sections of whole-side bone formation by the osseo-integrated implant at 30 days post-transplantation. Scale bar, 500 µm and 50 µm in the lower and higher magnification figures, respectively. Di, distal side; Ap; apical side; Me, mesial side; AB, alveolar bone; Imp, implant.
(F) Photograph, micro-CT images and HE section of removed an osseo-integrated implant attached to surrounding bone. Arrowheads show attached to bone tissue on the implant surface. Scale bar, 500 µm.

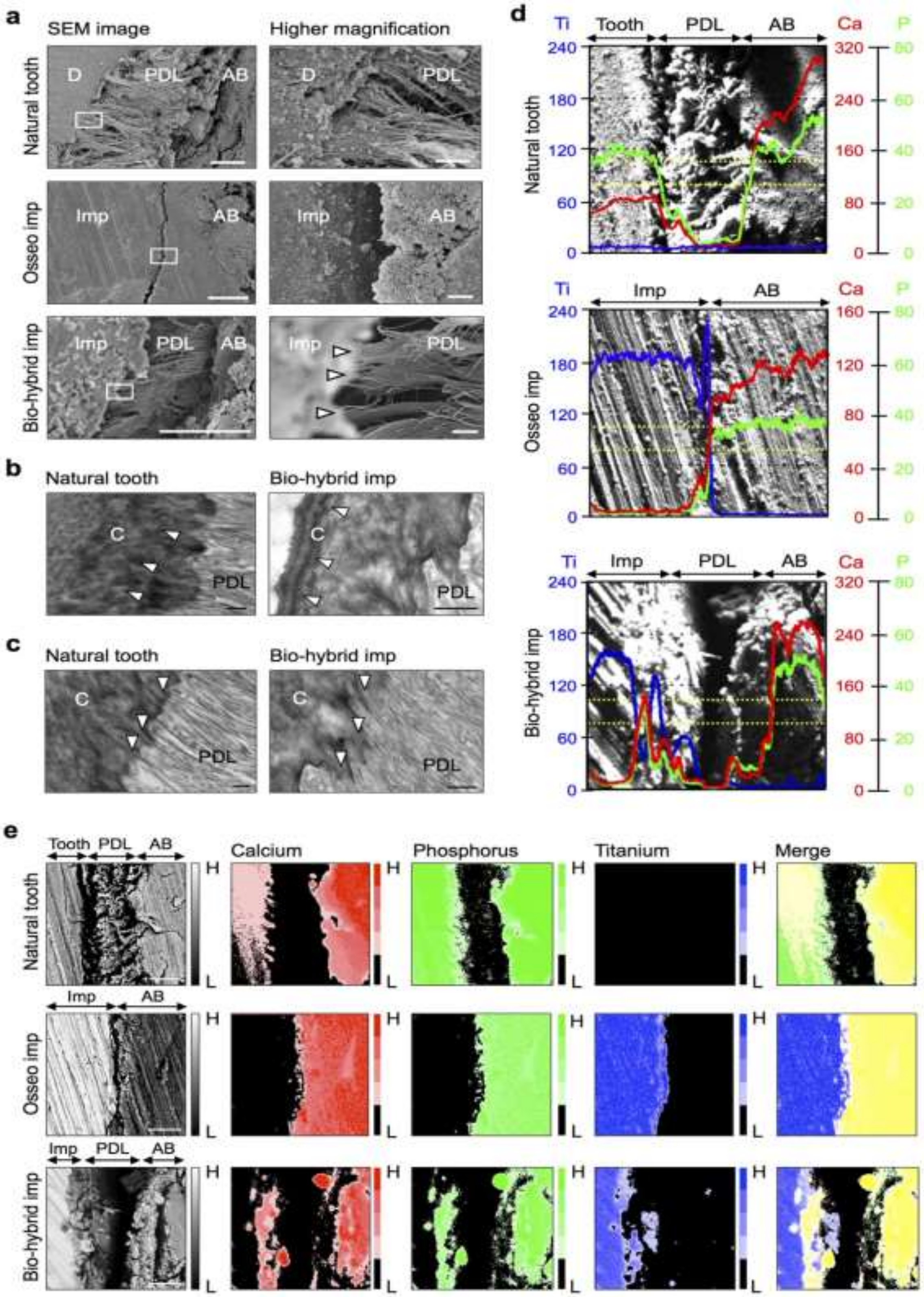


FIG. 2. Transplantation and Engraftment of the Functional Implant.

(A) Histological analysis of the natural mandible of the rat. Scale bar, 3.0 mm in the low-magnification HE figure and 100 µm in the high-magnification figure. AB, alveolar bone; BV, blood vessel; PDL, periodontal ligament.
(B) Photographs of an isolated PDL tissue (upper) and HE image of an isolated rat PDL (lower). Scale bar, 2.0 mm and 50 µm in the photographs and HE image.
(C) Micro-CT images of a functional implant in cross-section and frontal section during the processes of tissue remodelling and the connection between the recipient jaw bone and the implant surrounding the bone at transplantation Day 7, Day20 and Day 40. Scale bar, 500 µm.
(D) Histological analysis of a natural tooth (upper), an engrafted bone-attached implant without PDL tissue for control (middle) and an engrafted functional implant (lower) at 40 days post-transplantation was performed. HE and Azan staining are shown. Scale bar, 500 µm in the lower magnification (left column) and 50 µm in the higher magnification (centre and right column). D, dentin; C, cementum; AB, alveolar bone; PDL, periodontal ligament; Imp, implant; Imp SB, implant surrounding bone.

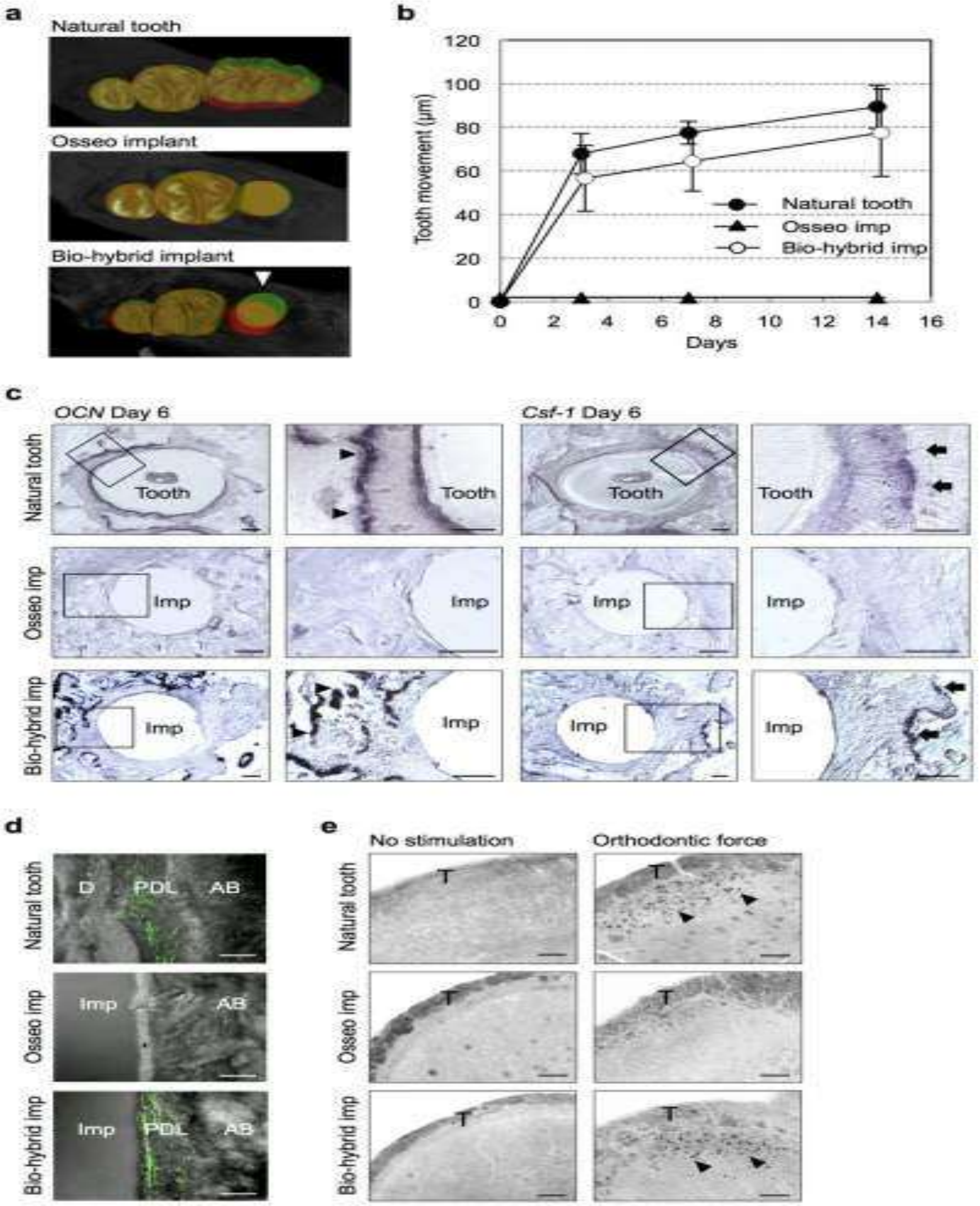


FIG. 3. A Functional Implant’s Response to Mechanical Stress

Sections of a natural tooth, bone-attached implant without PDL tissue and functional implant were analysed using in situ hybridisation analysis for OCN and Csf-1 mRNA at Day 7 of orthodontic treatment. OCN mRNA-positive cells (arrowhead) and Csf-1 mRNA-positive cells (arrow) are indicated. Scale bar, 100 µm.

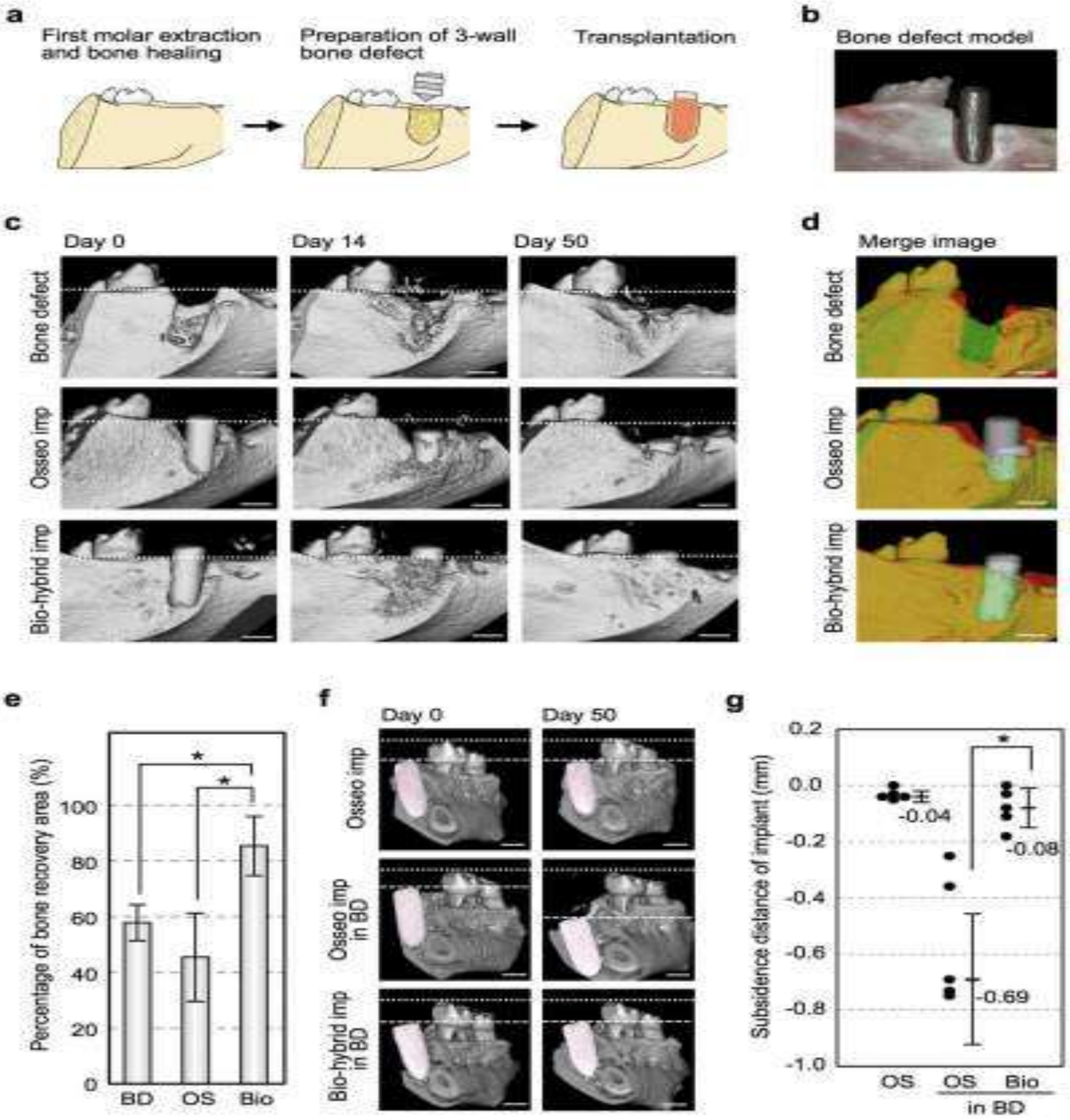


FIG. 4. Neural Function Analysis of an Engrafted Functional Implant

(A) Immunohistochemistry analysis of nerve fibres in the PDL of the functional implant using specific antibodies for neurofilament (NF; green). Scale bar, 50 µm. AB, alveolar bone; PDL, periodontal ligament; Imp, implant; Imp SB, implant surrounding bone.
(B) Expression of c-Fos immunoreactive neurons in the medullary dorsal horns of mice after 0 hours (no stimulation, control; left) and 2 hours of stimulation by orthodontic force (right). In the natural tooth (top), the bone-attached implant for control (middle) and the functional implant (bottom), the c-Fos protein was detectable after stimulation. Scale bar, 100 µm and 50 µm in the lower and higher magnification figures, respectively. T, spinal trigeminal tract.

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