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Enhancement of osseointegration of hydroxyapatite implants by Low-Intensity Ultrasound Irradiation

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ABSTRACT

Aim The purpose of this study is to investigate the enhancement effect on osseointegration of low intensity ultrasound for reinforcement of bone attachment of hydroxyapatite coated implants.

Materials and methods In this study, hydroxyapatite specimen were prepared, two kinds of experiments (simulation test and animal experiment) were performed.

Hydroxyapatite specimen were soaked in the simulated body fluid (SBF) as a simulation test, implanted rabbits iliac crest as in vivo test. After the irradiation of pulsed ultrasound wave for some planned time–periods, the surface of specimens were assessed and compared with non-ultrasound waving specimens (control group) by using Scanning Electron Microscope (SEM), Energy Dispersive Spectroscop (EDS) and X-ray diffraction.

Results SEM image and EDS showed that richer layer of bone-like hydroxyapatite covered specimens surfaces in ultrasound wave irradiation group as compared with control group. The measurement of mass of specimens also indicated the efficiency of ultrasound waves for hydroxyapatite formation. These results indicated low intensity ultrasound wave might promote the nucleation and crystallization of bone-like apatite on hydroxyapatite surfaces.

Conclusion This study suggested that the clinical application of ultrasound waving has a great potential for enhancement of osseointegration of hydroxyapatite dental implant through the activation of bone bonding mechanism on material surface.

KEYWORDS Low-intensity pulsed ultrasound waves (LIPUS), Osseointegration, Simulated Body Fluid, Hydroxyapatite.

INTRODUCTION

Nowadays, dental implants have become the most common treatment for replacing missing teeth and aim to improve chewing efficiency, physical health, and esthetics. The favorable clinical performance of dental implants has been attributed to their firm "osseointegration" as a direct contact between living bone and the surface of a load-carrying implant at the histological level introduced by Brånemark (1,2). In particular, Hydroxyapatite (HA; Ca10(PO4)6(OH)2) has an excellent biocompatibility and osseointegration properties, therefore it has been widely used as dental implants' coating material.

Considering the clinical view, early bonding between dental implant and living bone will accelerate the onset of activity of patients soon after an operation, with subsequent good long-term results. Therefore, several solutions have been tried to accelerate the osseointegration or the bone bonding to HA. Much has been learned about this concept and significant improvements on the design and surface of implants were done to eliminate the important challenges of implant dentistry and orthopedic surgery (3-5).

On the other hand, to enhance the osteogenesis and endogenous healing of bone fracture, low-intensity pulsed ultrasound (LIPUS) stimulation has been used in orthopedic surgery and dental surgery (6-9). Moreover, recent studies in the metal engineering field reported that ultrasound stimulation could accelerate the crystallization of metallic glasses (10,11).

To date, we have already studied and reported that osseointegration, or direct bone-bonding ability of bioactive titanium metal, was enhanced due to the induced hydroxyapatite formation on the surface using pulsed ultrasound radiation 1(2-14).

In the present study, for the purpose of dental clinical application using ultrasound stimulation, we investigate whether low-intensity pulsed ultrasound waves could accelerate osseointegration of hydroxyapatite experimentally by the simulated body fluid (SBF) and in vivo tests in the rabbit.

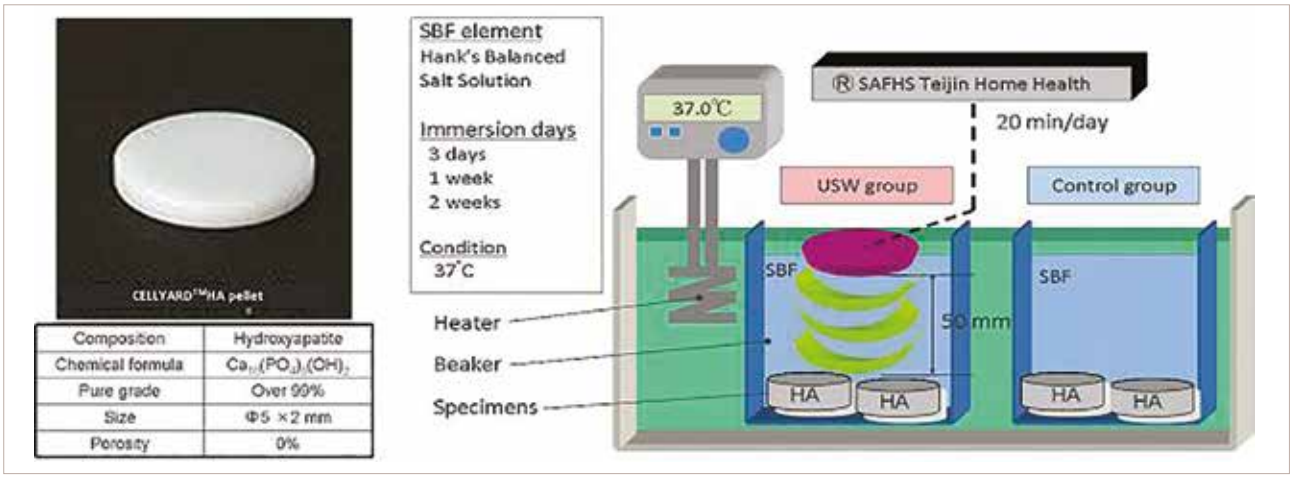

FIG. 1 Diagram of the pulsed ultrasound waving radiation on hydroxyapatite specimens in simulated body fluid (SBF).

MATERIALS AND METHODS

In the present study, in order to evaluate the effect of LIPUS on bone-bonding ability of hydroxyapatite ceramics (HA), pellets (99% more hydroxyapatite dense body; CELLYARDTM® , HOYA Co. Japan) were prepared as specimen.
Ultrasound radiation was applied by using Sonic Accelerated Fracture Healing System (SAFHS; Smith & Nephew, Memphis, TN, USA; Teijin Pharma, Tokyo, Japan). The treatment head module delivered ultrasound waves with 1.5 Hz sine waves, 200 µsec signal term, and spatial average intensity of 30 mW/cm^2 in both simulation test (in vitro) and animal experiment.
To evaluate the enhancement of osseointegration of the hydroxyapatite material by low-intensity pulsed ultrasound irradiation, two experiments, in vitro simulated experiment and in vivo tests using rabbits, were performed.

Simulated body fluid soaking test

As a basic bone-bonding ability test, the simulated body fluid (SBF) soaking method was performed as a simulation experiment according to Kokubo's studies in order to evaluate the apatite-forming ability of bio-active titanium and Bio-glass ceramics (15-17).
Hank's balanced solution (Lonza®; USA) was used as a SBF solution, and maintained at pH over 7.0 and 37°C and replaced every two days.
Ultrasound radiation was applied by using Sonic Accelerated Fracture Healing System (SAFHS; Smith & Nephew, Memphis, TN, USA ; Teijin Pharma, Tokyo, Japan). The treatment head module delivered ultrasound waves with 1.5 Hz sine waves, 200 µsec signal term, and spatial average intensity of 30 mW/cm^2.
HA samples (CELLYARDTM® pellet) were soaked in SBF and subjected to ultrasound stimulation for 20 min daily during the operation term for three days, one week, and two weeks, respectively. As a control, the same samples were left in SBF without ultrasound radiation under the same experimental conditions (Fig. 1).
After the above-mentioned processes were completed, the HA surface was subjected to scanning electron microscopy (SEM), energy dispersive spectroscopy (EDS) and X-ray diffraction (XRD), and these analyses were focused on the top surfaces, on which the ultrasound wave was directly radiated. Furthermore, to compare with the effect of ultrasound waves, the change of the sample's mass before/after the SBF treatment was measured.

In vivo experiment (animal experiment)

As an implant sample for animal experiment, the hydroxyapatite pallets (Ø5mm ×2mm) were cut into the sizes: 3 mm ×3 mm ×2 mm cuboid dice.
For an in vivo experiment, the bilateral iliac region of eight mature female rabbits (body weight 2.5-3.0 Kg) were used. Under intravenous anesthesia with Nembutal

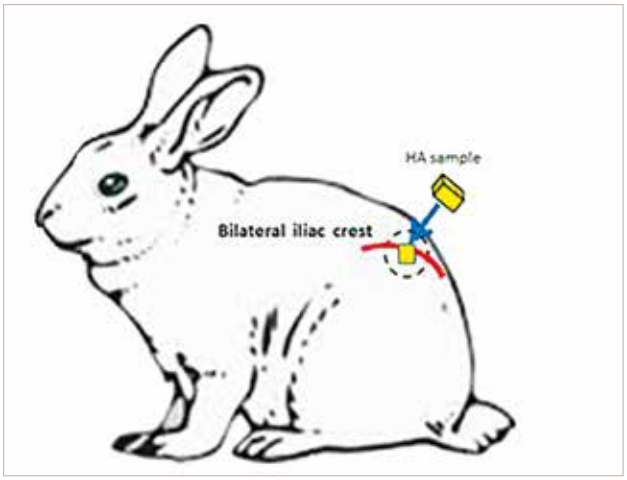

FIG. 2 Diagram of the present animal experiment.

(2-3 ml), the operation was performed using aseptic procedures. The implantation site in the iliac bone was exposed through an incision on the iliac crest, and blunt dissection of the muscle entheses. The periosteum was elevated at the implant insertion area, and a bone hole was made using a drill and file, and processed cuboid cavity to insert a cubic apatite specimen in each site. Figure 2 shows this surgical experiment. After implanting the specimen, the surgical area was washed, the overlying soft tissue was closed in layers.

After operation, one side of the iliac region of the rabbits was radiated with ultrasound waving by using Sonic Accelerated Fracture Healing System (SAFHS; Smith & Nephew) as a low-intensity pulsed ultrasound LIPUS stimulation group, another side was not radiated as a control group.

All rabbits were reared in cages with no postoperative immobilization, and sacrificed at four weeks after operation, HA samples were resected from the rabbits, the bone contact surface was examined by scanning electron microscopy (SEM), and energy dispersive spectroscopy (EDS). Furthermore, we tried to measure the HA-bone bonding strength by mechanical pull-out testing; however, in no sample the good interface between the bone tissue and HA without failure could be obtained because of the hard and brittle characteristics of HA materials.

All the above manipulations in the experimental process were in conformity with the Guideline for Animal Experiments, Institute for Frontier Medical Sciences, Kyoto University of Japan.

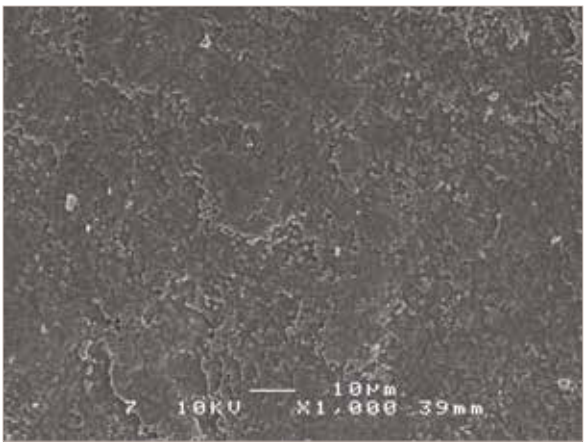

FIG. 3 SEM micrographs o the hydroxyapataite specimens surface before SBF soaking (1000 ×).

RESULTS

SEM observation *in vitro* test

Figure 3 shows the SEM images of the texture on the HA specimens surface before ultrasound radiation.

Figure 4 shows the SEM images of the texture on HA specimen surface of the LIPUS and control group. In

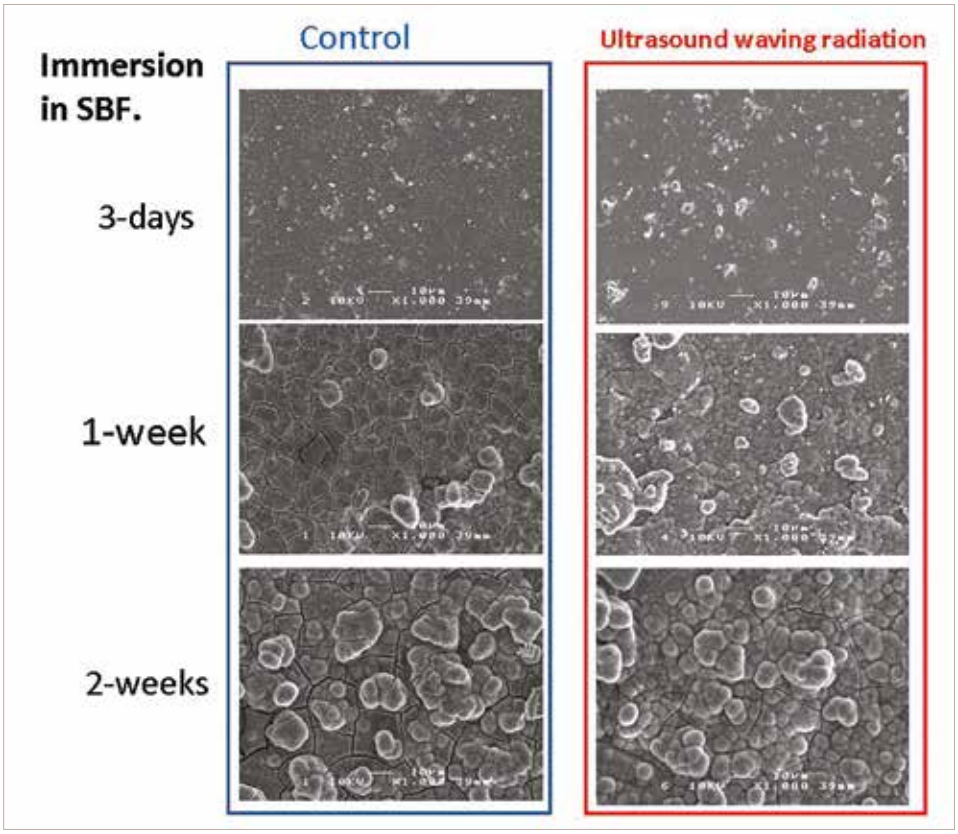

FIG. 4 SEM micrographs of the hydroxyapatite specimen surface after SBF soaking (1000 ×).

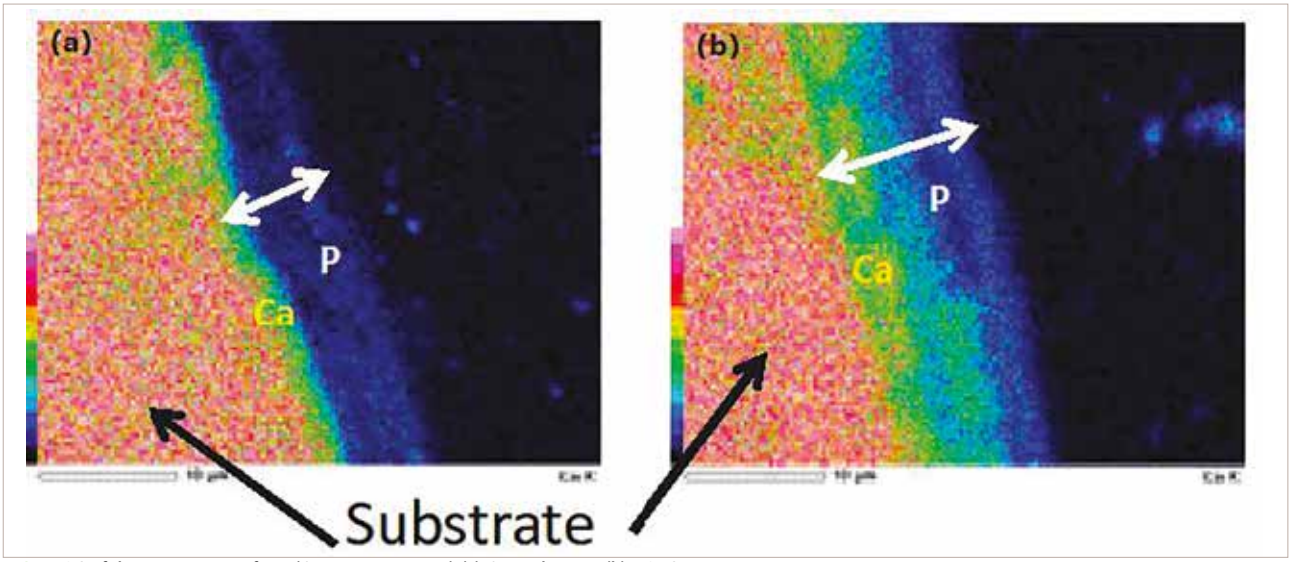

FIG. 5 EDS of the specimen surface (Cross section view) (a) Control group (b) LIPUS group.

both groups, morphological changes of the crystalline on the substrate surface have showed according to the soaking time. In the immersion experiments for 3 days, more minute particles showed on the ultrasound radiated surface as compared with the control group. Until one week, the morphology of the structure in the LIPUS group progressed to dendrite or needle-like crystalline structures, while the texture of the control group remained as small particles. By two weeks, the groups exhibited even greater differences in morphologies in terms of the crystallization on the substrates. The LIPUS group had changed into larger global grains, which indicates stable precipitate processes in terms of chemical equilibrium. These crystallization change on HA surface have demonstrated more active progress and growth in the LIPUS group.

EDS (energy dispersive spectroscopy) analysis

These compounds on HA surface were identified by EDS (energy dispersive spectroscopy) analysis.
Figure 5 compares the EDS spectra of the cross section view of the HA surface which were immersed in SBF for 2 weeks under ultrasound radiation (LIPUS group) and control groups.
As shown in figure 5 (a) and (b), Peaks of calcium (yellow) and phosphorous (blue) can be seen in the deposition area, confirming the presence of calcium-phosphate compound, USW group sample shows higher rich Ca and P peak intensity on the HA substrate.

XRD (X-ray diffraction) analysis

These compounds on HA surface were also identified by XRD (X-ray diffraction).
The XRD patterns of the layer on the HA surface after one week of the two groups are shown in Figure 6. Though the typical XRD pattern of hydroxyapatite was not observed, some peaks of hydroxyapatite were observed. Considering the existence of a calcium-phosphate compound coating confirmed by the EDS spectra, these data suggested a series of chemical processes in which the calcium and phosphorous ions were adsorbed and deposited onto the HA surface, and then transformed from calcium phosphate into crystalline of hydroxyapatite.

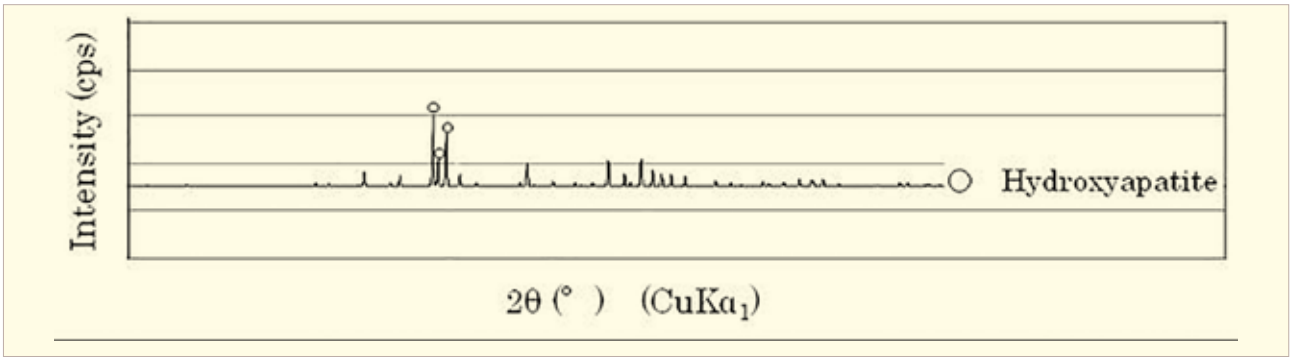

FIG. 6 XRD of the hydroxyapatite surface after soaking in SBF for 2weeks.

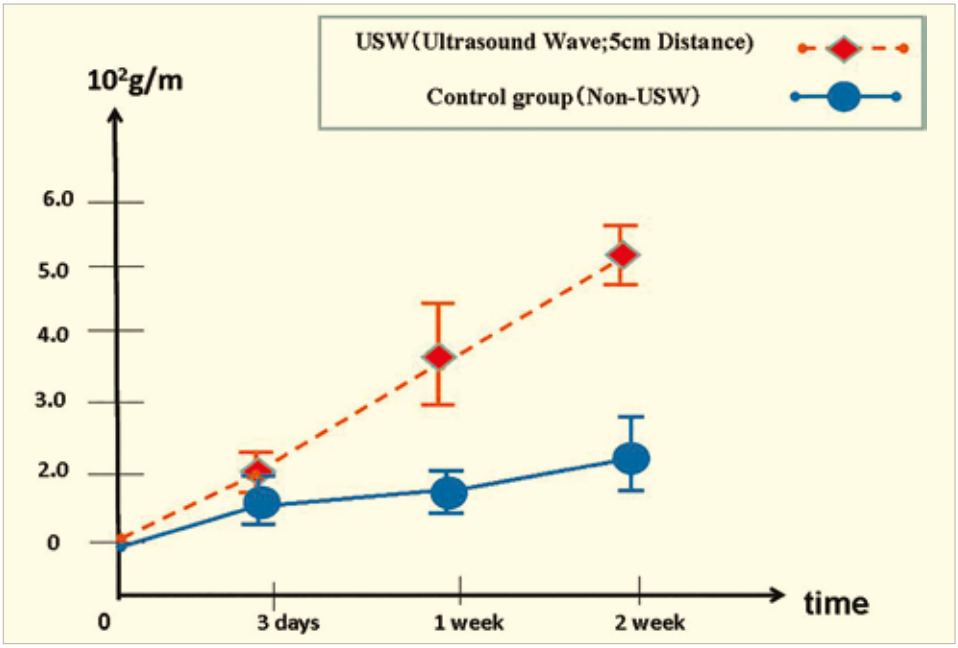

FIG. 7 Soaking time dependence of mass of P/Ca precipitation formation on hydroxyapatite (HA) specimen's surface (radiation distance: 50 mm).

Measurement of the mass of Ca-P crystallization

Figure 7 shows the measured sample mass change as a hydroxyapatite-like precipitation (calcium phosphate crystalline) before/after SBF soaking. In USW group, this crystalline showed significant increase as compared with the control group according to SBF soaking times ($p<0.05$).

In vivo animal experiment: Macro appearance of the animal specimens

Figure 8 shows the macro-appearance of HA implant on rabbits iliac crest. Neither inflammation nor infection signs were observed in this area.

All HA implants were fixed in the iliac crest bone, many sample of the control group showed more weak attachment, easily disconnected from the bone tissue. Many samples had not enough interface between the bone and HA in order to cut out from rabbits, appropriate sample for the mechanical pull-out testing to measure the HA-bone bonding strength that could be obtained.

SEM observation of the implant surfaces

Figure 9 shows the SEM micro-appearance of the surface of HA sample implanted in rabbits. As shown for the SBF soaking test (Fig. 4), the morphological change of the crystalline on the HA surface was observed. This finding indicated the precipitation and growth of bone-like hydroxyapatite on HA surface, the crystalline change on

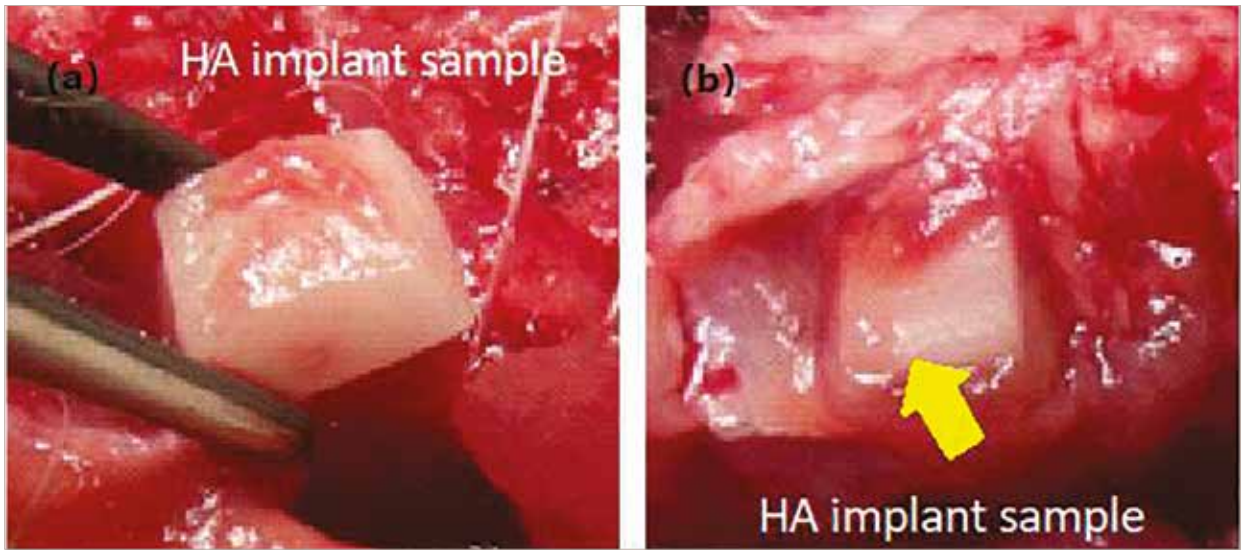

FIG. 8 Macroscopic appearance of HA implant in rabbits iliac bone after 2weeks. Control group (a), and low-intensity pulsed ultrasound (LIPUS) group (b).

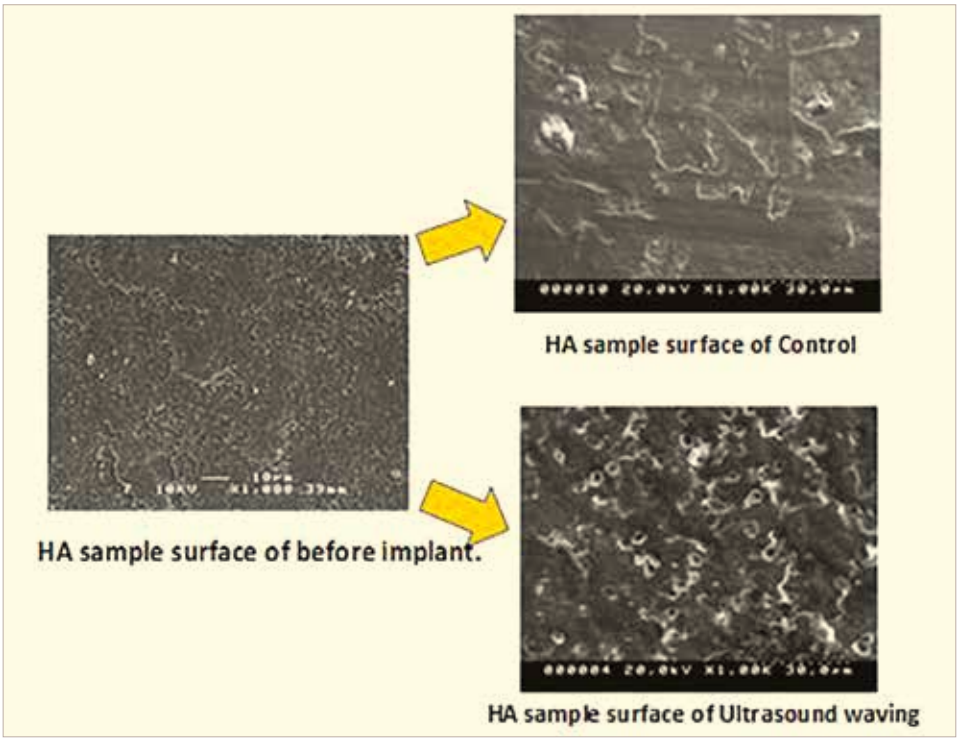

FIG. 9
SEM image of the hydroxyapatite implant surface of control group and LIPUS group. (×1000)

substrate showed more active progress and growth in the LIPUS group.

EDS analysis of the implant surfaces

The precipitation layer on the surfaces of HA implant were also observed by energy dispersive spectroscopy (EDS). Figure 10 shows the micro appearance of implanted HA sample in LIPUS and control groups. As shown in the figures, this precipitation indicated the composition of rich phosphate (P) and calcium (Ca) as well as bone tissue. The carbon (C) in EDS images represented a composition of organic substance such as collagen fiber etc. from bone tissue, which suggested a biological reaction to osteo-conductive biomaterial in vivo.
These findings indicated the good bone bonding condition between the HA surface and bone tissue, the existing of more rich calcium-phosphate crystal layer in the specimen of LIPUS group could be related to the enhancement of osseointegration by ultrasound waving.

DISCUSSION

The simulation method using SBF soaking in this study is well known as an in vitro assay for evaluation of bone-bonding ability of biomaterials, and has been already used widely in studies regarding the osteoconductivity (18-21).
According to Kokubo, who developed SBF, the osseointegration of biomaterials is mainly due to three step mechanisms, at first the calcium-phosphate precipitate and grows on material surface as bone-like apatite in biological tissue, this apatite layer secondary progress the bonding to the apatite of bone tissue by the mechanism of crystal structure in vivo, and the process of the biological bonding to natural bone tissue follows. Therefore, in vivo bone-bonding ability of bio-active material depends on the first apatite formation on the surface. When this biomaterial is soaked in SBF, the nucleation and growth of calcium phosphate compounds from SBF occur on this surface layer. Therefore, the apatite formation on the surface in SBF is useful for the osseointegration ability of the biomaterial. In fact, it has been already confirmed that in vitro results in SBF concerning the bone-bonding ability of bio-active materials correspondent to the results for osteointegration in vivo (15,21). As a result, the increase of calcium-phosphate precipitation including partial hydroxyapatite crystalline by ultrasound irradiation was confirmed. Morphological observation at SEM in the early phase of SBF soaking exhibited more nuclei on the HA surface in the USW group than in the control group. This finding implies that the enhanced crystallization process due to the influence of ultrasound waves occurs at the initial state of nucleation and growth of calcium phosphate compounds. The microstructural feature of accelerated calcium-phosphate crystalline at 1 week under the USW showed branch-like morphology, i.e. the dendrite type, which indicates rapid crystalline formation under poor latent heat transfer (22,23). This fact indicated that the partial concentration and interatomic potential of the Ca and PO4 ions in SBF can be changed by ultrasound microvibration, and accelerated to the nucleation and deposition of ions, resulting in

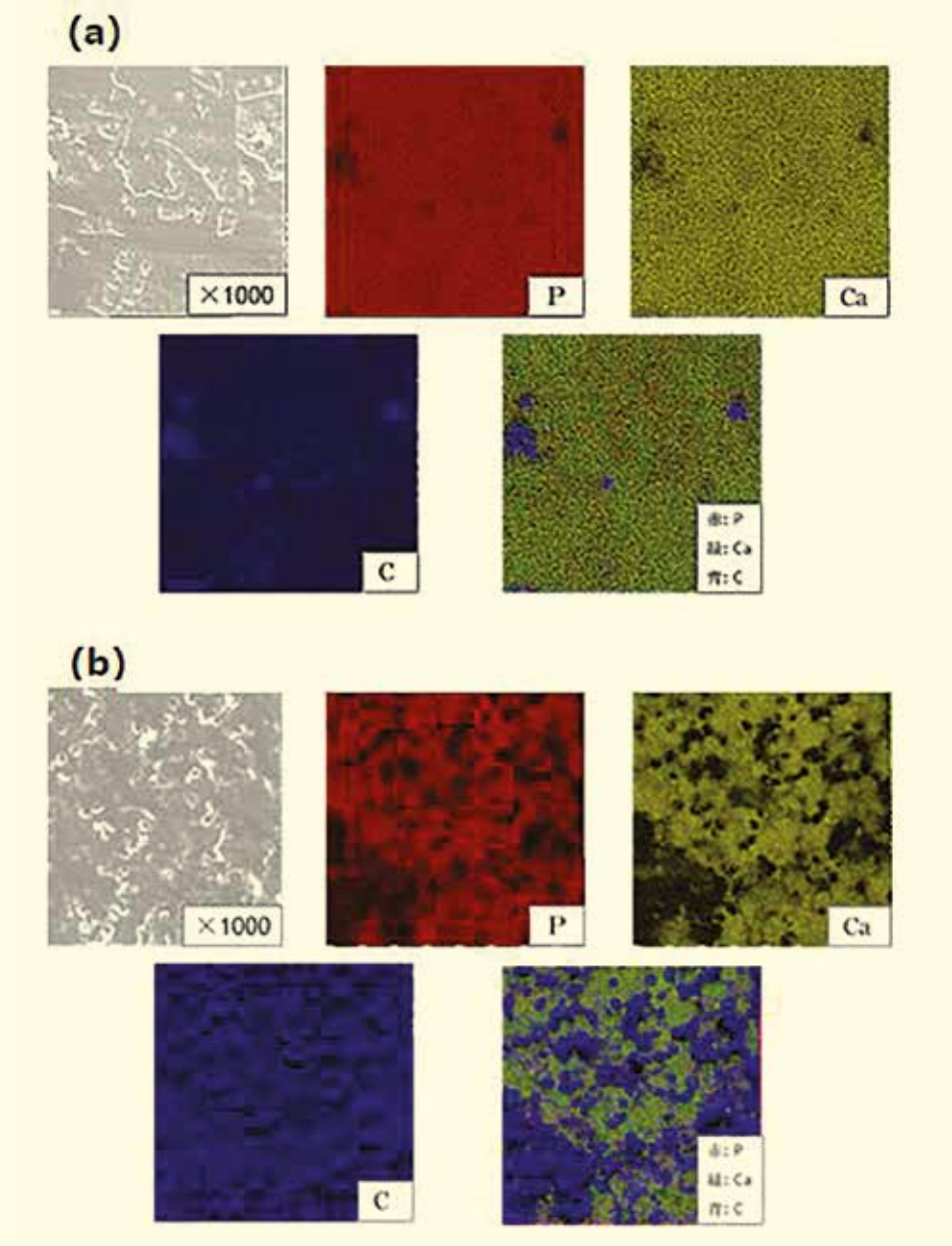

FIG. 10 EDS of the hydroxyapatite implant surface from rabbits (a) Control group (b) LIPUS group.

the crystallization of calcium phosphate on activated titanium surface layer. Cavitation by ultrasound waves might also contribute to the formation of the apatite layer by means of the microfracture of calcium-phosphate and the circulation of new Ca and PO4 ions of SBF on the titanium surface. These environmental changes will accelerate further changes of interatomic potential of surrounding atoms, and deteriorate the thermal stability of calcium-phosphate phase versus Ca and PO4 ions. Tanaka has already reported an increase of degrees of freedom and number of crystal orientations for atomic pileup by thermal vibration concerning the relationship between the dendritic crystal and ultrasound vibration (24). According to this report, the rapid crystallization caused by ultrasound waves is considered to enhance the nucleation process rather than the growth stage. The similar ultrasound-induced crystallization has been reported in the metallurgy field; however, its detailed mechanism is still unclear (10,11,25).

Our result also suggested that the initial nucleation and deposition of calcium phosphate on activated titanium surface layer could be very important for the enhancement of the crystallization by ultrasound waving.

Following the SBF simulation tests, some analysis in animal experiments using rabbits also showed more rich apatite layer formation on the surface in USW irradiated group. Although the mechanical strength between the bone and HA implant in this study could not be measured, this in vivo result strongly suggests the possibility of the enhancement of osseointegration by ultrasound irradiation.

In vivo mechanism of the ossointegration enhancement by low-intensity ultrasound irradiation is more complicate and difficult to elucidate because of the biological osteogenesis function by osteoblast and fibroblast cells etc.

Recently, Abdulhameed et al. have also reported in a clinical research that low-intensity pulsed ultrasound irradiation might enhance the osseointegration (bone-bonding) by means of X-ray radiographic evaluation in bone tissue around implant (26), the authors speculated that LIPUS promotes osseointegration, bone attachment to implant due to the bone formation and healing in dead space around dental implants by inducing, triggering and provocating the osteoblast cells.

Our in vivo study also supported this report, in addition, our SEM and EDS data indicated that low-intensity ultrasound irradiation activates not only biological pathway for bone tissue but also bone-apatite precipitation on the implant surface as a mechanism of the ossointegration enhancement

Although LIPUS therapy has been shown to enhance the osteogenesis and is widely used as clinical treatment for complicated bone fracture in the orthopaedic surgery field, little is known about the mechanism of action of ultrasound in spite of many experiments and investigations on this subject. Therefore, further assessment regarding the mechanism and clinical optimum conditions of ultrasound waving therapy for ossointegration will be necessary, however, the present study strongly suggests that low-intensity pulsed ultrasound waving has potential as an excellent clinical application for the osseointegration between the HA coating implant and maxillary bone in dental surgery.

CONCLUSION

This study suggested that the clinical application of low-intensity ultrasound irradiation has a great potential for enhancement of osseointegration of hydroxyapatite dental implants by promoting the mechanism of nucleation and crystallization of bone-like apatite on implant surfaces.

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Conflict of interests

The authors declare no potential conflict of interests.

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REFERENCES

1. Brånemark PI, Hansson BO, Adell R, Breine U, Lindström J, Hallén O, Ohman A . Osseointegrated implants in the treatment of the edentulous jaw. Experience from a 10-year period. Scand J I Plastic and Reconstr Surg Suppl 1977;16: 1-132.
2. Brånemark P I. Osseointegration and its experimental background. J Pros Dentistry 1983; 50: 399-410.
3. Smeets R, Stadlinger B, Schwarz F, Beck-Broichsitter B, Jung O, Precht C, Kloss F,Alexander Gröbe A, Heiland M, Ebker T. Impact of dental implant surface modifications on osseointegration . BioMed Res Inter 2016 ;1-16.
4. Thomas KA, Kay JF, Cook SD, Jarcho M. The effect of surface macrotexture and hydroxyapatite coating on the mechanical strength and histologic profiles of titanium implant materials. J Biomed Mater Res 1987; 21: 1395-1406.
5. De Andrade MD, Sader MS, Filgueiras MRT, Ogasawara T. Microstructure of ceramic coating on titanium surface as a result of hydrothermal treatment. J Mater Sci Mater Med 2000 ; 11 : 751-55.
6. Heckman JD, Ryaby JP, McCabe J, Frey JJ, Kilcoyne RF. Acceleration of tibial fracture-healing by non-invasive, low intensity pulsed ultrasound. J Bone Joint Surg (A) 1994 ; 76 : 26-34.
7. Kristiansen TK, Ryaby JP, McCabe J, Frey JJ, Roe LR. Accelerated healing of distal radial fractures with the use of specific, low-intensity ultrasound. A multicenter, prospective, randomaized, double-blind, placebo-controlled study. J Bone Joint Surg (A) 1997 ; 79 : 961-73.
8. Tanzer M, Kantor S and Bobyn JD. Enhancement of bone growth into porous intramedullary implants using non-invasive low intensity ultrasound. J Orthop Res 2001;19 : 195-199.
9. Rego EB, Takata T, Tanne K and Tanaka E. Current status of low intensity pulsed ultrasound for dental purposes. Open Dent J 2012; 6: 220-225.
10. Ichitsubo T, Matsubara E, Kai S, Hirano M. Ultrasound-induced crystallization around the glass transition temperature for Pd40Ni40P20 metallic glass. Acta Mate 2004 ; 52 : 423-429.
11. Ichitsubo T, Matsubara E, Anazawa K, Nishiyama N. Crystallization accelerated by ultrasound in Pd-based metallic glasses. J Alloy Compounds 2007 ; 434-435 : 194-195.
12. Kobayashi M, Noda K, Tatematsu N. Preliminary in vitro study on Enhancement of Bone-like Hydoxyapatite formation on bioactive titanium alloy by low-intensity pulsed ultrasound waving for early bone bonding. J Biomech Sci and Eng 2010 ; 4 : 449-460
13. Kobayashi M Study on enhancement of Osseointegration of the bio-active titanium implant by low intensive Ultrasound wave. (Part1: Simulated body fluid soaking test). Int. J Biomed Eng Sci (IJBES) 2017 ; 4 : 1-10.
14. Kobayashi M, Shiraishi M, Kinoshita M. Study on enhancement of Osseointegration of the bio-active titanium implant by low intensive Ultrasound wave. (Part2: In vivo Experiment). Int J Biomed Eng Sci (IJBES) 2018; 1 : 1-10 .
15. Kokubo T, Takamada H. How useful is SBF in predicting in vivo bone bioactivity? Biomaterials 2006 ; 27: 2907-15.
16. Kokubo T, Miyaji F, Kim HM, Nakamura T. Spontaneous formation of bonelike apatite layer on chemically treated titanium metals. J Am Cerami Soc 1996 ; 79 : 1127-1129.
17. Kokubo T, Yamaguchi S. Novel bioactive titanate layers formed on ti metal and its alloy by chemical treatments. Materials 2010; 3: 48-63.
18. Yamaguchi S, Akeda K,Murata K,Takegami N,Goto M, Kokubo T. Chemical and Heat Treatments for Inducing Bone-Bonding Ability of Ti-6Al-4V Pedicle Screw. Key Eng Mate 2015 ; 631: 225-230.
19. Mukai H, Kobayashi M The Effect of Low-intensive Pulsed Ultrasound Waving on Hydroxyapatite in Simulated Body Fluid Proceed. of IEEE 11th Inter. Confer. on Bioinf. Bioeng (BIBE) IEEE Computer Society 2011; Taipei : 125-8.
20. Sato T, Shimizu Y, Odashima K, Sano Y, Yamamoto A, Mukai T, Ikeo N, Takahashi T, Kumamoto H. In vitro and in vivo analysis of the biodegradable behavior of a magnesium alloy for biomedical applications. Dent Mate J 2018 ;1: 1-11.
21. ZhaoW, Michalik D, Ferguson S, Hofstetter W, Lemaître J, von Rechenberg, Bowen P. Rapid evaluation of bioactive Ti-based surfaces using an in vitro titration method. Nature commun 2019 ; 10 : 2062.
22. Hurle D.T.J. Handbook of crystal growth. North Holland;1993.
23. Teraoka Y, Saito A, Okawa S. Ice crystal growth in supercooled solution. Int J Refrigeration 2002 ; 25 : 218-225.
24. Tanaka A, Sawada K. The Effect of the Pulse ultrasonic waves in the dendritic crystal growth. Bussei.Kenkyu 1989 ; 52 : 430-434 (in Japanese).
25. Ichitsubo T, Matsubara E, Anazawa K, Nishiyama N, Naito M, Hirotsu Y. Low-temperature crystallization caused by ultrasound in Pd42.5Ni7.5Cu30P20 and Pd40Ni40P20 bulk metallic glasses. Mate Sci Eng 2006 ; A442 : 273-277.
26. Abdulhameed EA, Enezei HH, Omar M, Komori , Sugita Y, Hegazy FA, Ari S, Maeda H, Alam MK The Effect of Low Intensity Pulsed Ultrasound Therapy on Osseointegration and Marginal Bone Loss Around Dental Implants. J Hard Tissue Bio 2017; 26 :323-330.