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Original

Qualitative study of the New Bone formation Surrounding the Ti-implant by FTIR and Polarizing Microscope

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Abstract: Recently, dental implants have gained much attention for a great number of researchers in dental application. Until now, there are few reports about the properties and metabolism related to the bite force response of the newly bone formed surrounding the implants, which is poorly understood. For that reason, the purpose of this study was to elucidate the new bone formation surrounding the implants and its process of maturation by Micro-CT, polarizing microscope and FTIR analysis. Thus, the implants were inserted in the tibia of rabbits. After 4 and 8 weeks of surgical procedure, three-dimensional image of the implant and bone of non-decalcified samples was observed by Micro-CT analysis; bone structure and its maturity were seen by polarizing microscope; and finally, the PO_4^{3-}, CCO_3^{2-} and Amide I elements included in the interface bone-implant and in the new bone during the course time were examined by FTIR analysis. The results of this study, analyzed by this 3 methods, demonstrated that: 1) It was observed a qualitative difference in the new bone formation and the existing bone at 4 and 8 weeks of implantation; 2) There was also a qualitative difference in the newly bone formed at 4 and 8 weeks; 3) The maturation of the new bone started from the exhibiting bone; and 4) None of the methods used in current study destroyed the samples, and reproducibility results were obtained. In conclusion, we suggest a possible qualitative evaluation of new bone formation surroundings the Ti implant.

Key words: XRD, FT-IR, Polarizing microscope, New bone formation, Ti implant

Introduction

Titanium implants and its alloys are widely used in orthopedic and dental applications. However, some implants are not bioactive enough to form a direct bond with bone, which sometimes translates into a lack of osseointegration into juxtaposed bone that might lead to long term implant failure, where the cause is not always clarified. The reason is because there are many negative factors that affect the stability of the implant in clinical trials. Recently, the elucidations of unknown negative factors which alter the stability of the implant are required because the implants became a popular alternative as treatment.

Moreover, the risk of the rejection of implant cannot eradicate. The cause of the delay to evidence those negative factors related to the implant treatment is for the complexity of properties, metabolism and mechanism of bone in hard tissue research. Until now, it has been mainly reported a quantitative studies of the new bone formed surrounding the implant, and bone-implant interface[1-2)]. In contrast, few studies have focused in the qualitative of the newly bone attaching the implant.

It is well known that the bite force of an adult with complete dentition is approximately 5-40 kg[3-4)]. Because of this, we believe that dental prostheses can be the same. In order to lead a long term stability of the implant under a harsh condition is essential to acquire enough osseointegration; the minimum requirement period of time is 3 months in mandible and 6 months in maxilla[5)], which is not yet well established. In addition, the mechanism of bone formation has different stages. Thus, the developing bone tissues from its initial stage to mature bone pass for various maturation processes. So far, the amount of the new bone and the interface ratio was measured; and might the hard tissue surrounding the implant have enough calcification to be able to support the Ti

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implant similar to the existing bone?. Isn't that the bone tissue includes various maturation levels being all of them summarized in one word called new bone? The early stage of bone formation becomes an important key to obtain enough osseointegration. Especially, there is a tissue that supports directly the implant after operation, which become a crucial element. In order to evaluate the crystallinity in the existing bone and in the new bone, it was used a polarizing microscope, Laboratory for Electron Beam Research and Application Institute of Quantum Science, Nihon University-Parametric, X-ray radiation (LEBRA-PXR), microscope Fourier transform infrared (FTIR), Micro-XRD (Micro- X- ray Diffractometer).[6] Moreover, Nakada et al. reported that X-ray Photoelectron Spectrometer detected the difference about the element contents of existing bone and new bone. [7] On the other hand, it has not still clarified about the qualitative change of the new bone in relation with the position of new bone, implant and cortical bone. Until now, the crystal analysis of the hard tissue is not clear because its low crystallinity with a complex organic and inorganic structure. This could be the reason why there are a few researches investigating the qualitative difference of the new and existing bone. However, even when the prediction of early increase of weight raises, it is possible to say that estimation of new bone is very important item same as the measurement of bone amount

The main components of the bone are collagen fiber as an organic component, and hydroxyapatite as an inorganic component. It is well known that hydroxyapatite is one kind of calcium phosphate composed of $Ca_{10}(PO_4)_6OH_2$. But actually, various minuscule amount of molecules and elements are replaced Ca^{2+},PO_4^{3-}, OH^- for CO_3^{2-}, Na^+, Mg^{2+} in the apatite in vivo. On the other hand, CO_3^{2-} is included in the bone tissue in approximately at 7% producing a distortion in the ideal crystal structure [9].Therefore, hydroxyapatite included in the bone express PO_4^{3-}; CO_3^{2-} and collagen influencing in the distortion of hydroxyapatite crystallinization express Amide I; for that reason, we believe that an estimation of the qualitative difference of bone of them may be performed by FT-IR analysis.

The purpose of the present study was to analyzed three-dimensional image of the implant and the bone of non-decalcified samples by Microcomputed tomography (Micro-CT); bone structure and their maturity by polarizing microscope; and finally, the PO_4^{3-}, CO_3^{2-} and Amide I elements included in the interface bone-implant and in the new bone during the course time were examined by FTIR analysis.

Materials and Methods

1. Animals

The procedure for the care and killing of the animals was in accordance with the experimental animal committee approval agreement of the Matsuo Dental School, Japan University (approval number 07-0016). In this study, fourteen male 18-weeks-old rabbits (New Zealand White Rabbit from Sankyo Lab service, Japan) of approximately 3 kg were used. The animals were housed in a rabbit gauge and acclimatized with standard conditions (temperature: 23±1°C; humidity: 50±1%) with free access to water and food pellets (rodent diet no. RC4).

2. Materials

The chemical composition of Ti alloy was (wt%): Ti15, Zr 4 and Nb 4 (Ti-15-4-4). Until now, Ti-15-4-4 implants showed a great dynamic quality, corrosion fatigue strength, corrosion resistance and cell biocompatibility. For that reason, Ti alloy is expected to be a future therapeutic strategy for medical application[10-11].

In the present study, we implanted Ti-15-4-4 (cylinder-shaped, 2.8 mm x 7 mm) in the tibiae of rabbits. Two kind of treatment (Type 1：Acid etching + NAF acid， and Type 2：Acid etching + NaF acid + Simulated body fluid, SBF) previously described by Prof. LeGeros, R. Z and Prof. LeGeros J. P from New York University College of Dentistry, Dept. Biomaterials and Biomimetics were performed on the surface of the implants. We used mirror-polished implant surface as control group for surface treatment group

3. Surgical procedure

The surgical procedure was described previously by Tanaka et al.[12]. The animals were intravenous anesthetized with 2mg/kg of ketalar. After that, bone defects of 20 mm were made in the tibiae of rabbits. We used (IMPLANTOR-S® ：kyocera, Japan) for insertion of the implants irrigated with saline solution at 800 times/minutes (gear ratio 1:16) high speed times/minutes. The treated groups: treated group-type1 (surface of the implants treated with Acid etching + NAF acid) and treated group-type 2 (surface of the implants treated with Acid etching + NaF acid + SBF) and untreated group (mirror-polished implant surface) as control were implanted in the left and right tibia of rabbits. After surgery, non-mobility of them was examined.

4. Preparation of non-decalcified specimens

The animals were sacrificed with an ear intravenous overdose of pentobarbital natorium (nembutal®: dainippon seiyaku, Japan) after 4 and 8 weeks of surgery. The specimens were removed and fixed with 10% formalin and examined by Micro-TC analysis. Then, the sampled were dehydrated with 70~100 % ethanol, 100% acetone and finally embedded in resin (osteoresin embedment kit: Wako Pure Chemical Industries, Japan) placing them parallel to the major axis. The specimens were then cut at 100 ìm with a diamond blade (isomet: buhler, usa).

5. Evaluation of the samples

Micro-CT analysis

The specimens were removed after 4 and 8 weeks of the

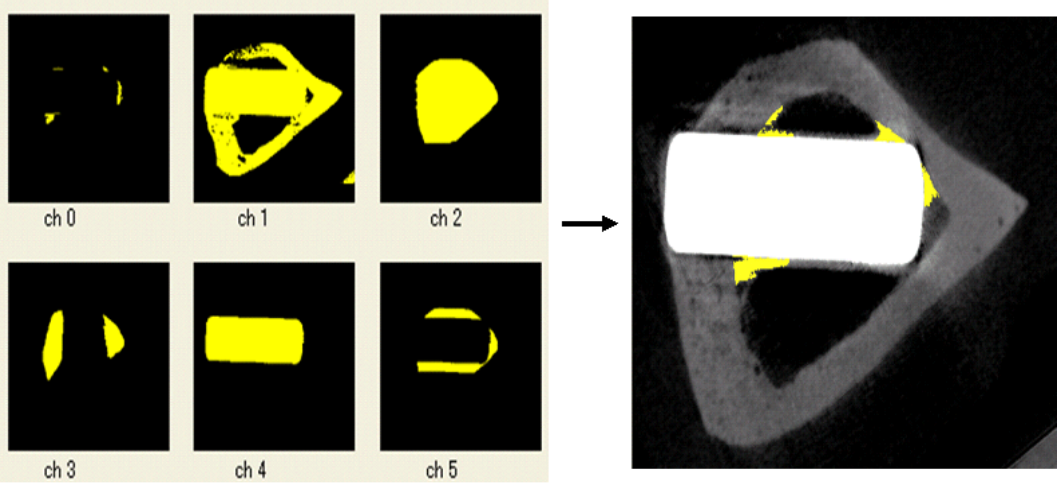

Fig. 1•Fig.1. Volume of the new bone detected by Micro-CT analysis
Total Volume (TV) = circumference 500μm of the implant ∧ marrow space = ch 5 New Bone Volume (BV) = ch 1 ∧ ch 3 ∧ ch 5

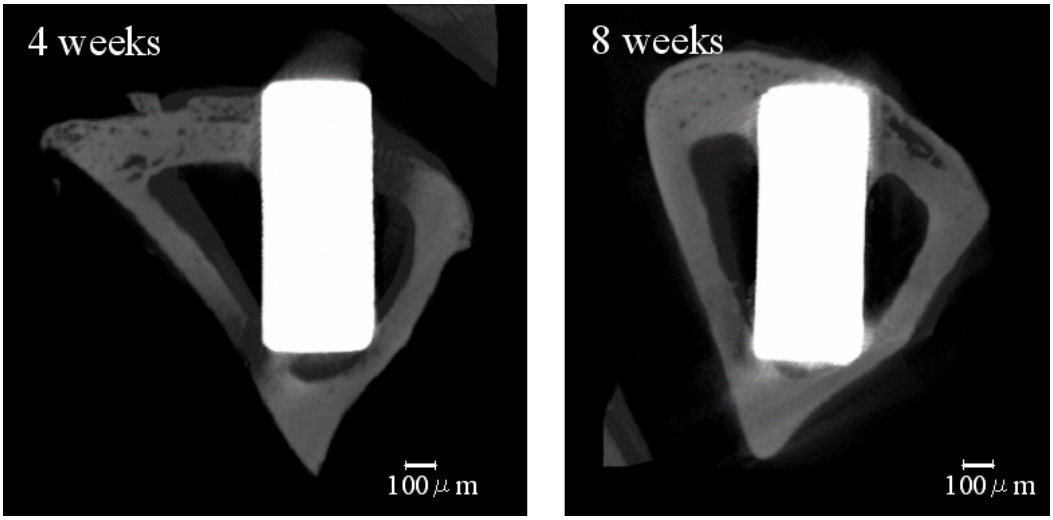

Fig.2. Micro-CT image of treated group-type 2 after 4 and 8 weeks of implantation.

operation, fixed with formalin solution and examined by Micro-TC analysis (R_mCT, Rigaku Corporation, Japan). The images were taken at 90kv, 85iA, 4X of magnification for 2 minutes of exposition. After that, the volume of the new bone surrounding the implant observed in Micro-CT image was measured using analytic software (3D BON, RATOC System Engineering, Japan). The volume of the new bone formed was obtained as follows:

Finally, differences among the results of the different groups were analyzed using Student's T test, with $p < 0.05$ considered as statistically significant.

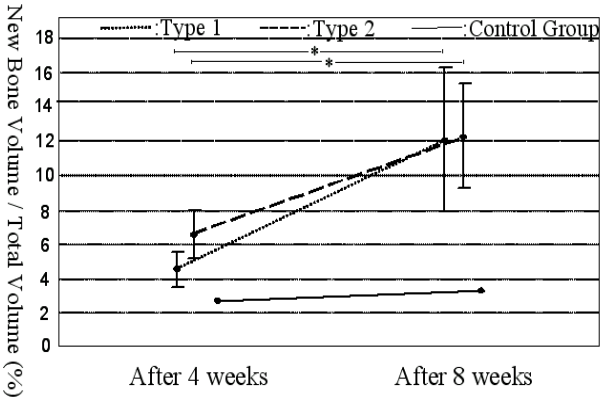

Fig.3. The ratio of the volume of new bone in the circumference 500im of the implant. The significance of differences between Type 1, 2 was examined by Student's T test (*: P<0.05).

Polarizing microscope analysis

The hard tissue of non-decalcified specimens contained both organic and inorganic components. For that reason, exhibit characteristic polarized light image as well as interference image due to double refraction of those components in the polarizing

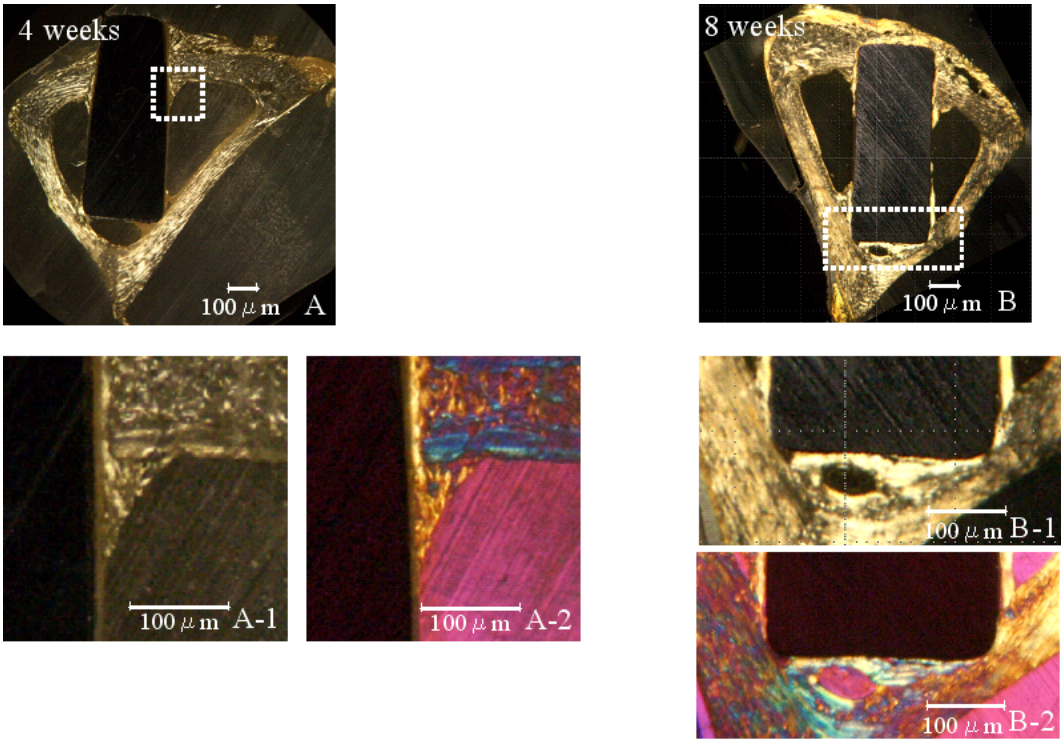

Fig. 4. Treated group- type 2 after 4 and 8 weeks of implantation observed by polarizing microscope analysis. A, A-1, B and B-1 show cross polarizer method crossed nicol method; A-2 and B-2 shows sensitive color plate method

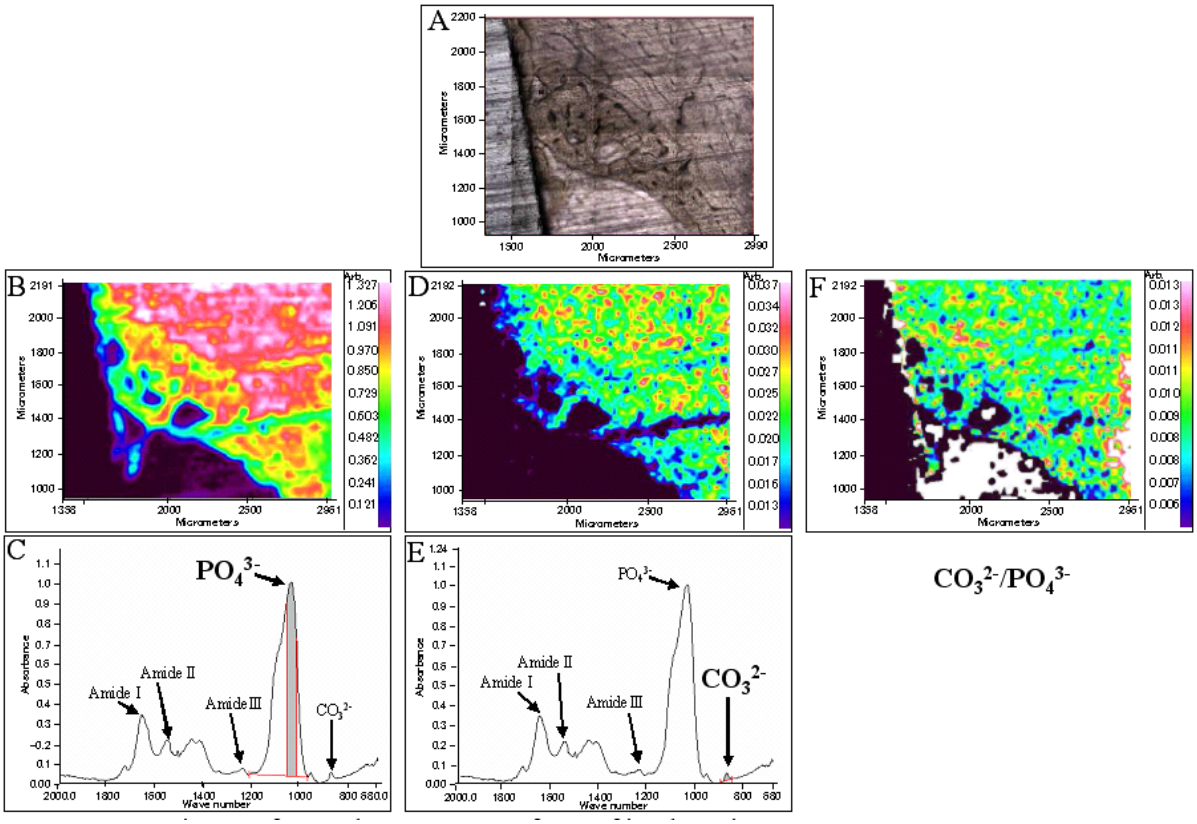

Fig.5. FT-IR image of treated group-type 2 after 4 of implantation. A: Measurement range, B: PO_4^{3-} distribution, C: PO_4^{3-} absorption band, D: CO_3^{2-} absorption band, E: CO_3^{2-} absorption band, F: CO_3^{2-}/PO_4^{3-} ratio of distribution

microscope[13]. For this observation, it was used polarizing microscope (OPTIPHOTO2-POL®: Nicon, Japan) with parallel nicol method, crossed nicol method and sensitive color plate method (Gypsum Plate)

FT-IR analysis

In order to investigate the distribution of the chemical component of the different groups, FTIR analysis was performed.

For this examination, we made a reflection image using Spotlight 400 (PerkinElmer, Inc.USA). We confirmed the correct area of the newly bone formed from the polarizing microscope view of the different groups at 4 and 8 weeks.

Analysis of the frequency area：4000~680cm^{-1}

(In this study, it was excluded the analysis at more than 2000 cm^{-1} because enters an artifact of measurement,)

Addition time：2 times

Resolution ：4cm^{-1}

Conversion data：K-K conversion, PCA analysis

Image pixel size：25ìm

Results

1. Micro-CT analysis

We showed 3D image in thin sections evidencing new bone and Ti implant. The figure 2 shows Micro-CT images revealing presence of cortical bone, Ti-implant and vessel canal system. The amount of the new bone from 4 to 8 weeks is observed in the figure 3.

Treated group

In this experiment, the implants inserted in the tibiae of rabbit were touching the upper and lower side of cortical bone. The treated group showed new bone formation from the upper and lower side of the existing bone along the implant surface to the center at 4 and 8 weeks. Moreover, the bone-implant interface was clearly detected. The radiopacity of the newly bone formed was increased from 4 to 8 weeks. On the other hand, the volume of the new bone increased significantly from 4 to 8 weeks in treated and control groups. However, there was no significant difference in new bone amount in the treated group- type A and B.

Control group

The control group demonstrated scanty formation of new bone, which was only seen attaching the existing bone in the upper side of the implant at 4 and 8 weeks; in the lower side, the implant was in contact with the existing bone without new bone formation. However, an increase of new bone from 4 to 8 weeks was revealed.

2. Polarizing microscope examination

Figure 4 shows clearly the orientation of collagen fibers by sensitive color plate method and cross polarizer method. We could also observe some overlapped lamellae structure in the existing bone that is common at 4 and 8 weeks either in treated group as well as control group. The lamellae structure of bone detected

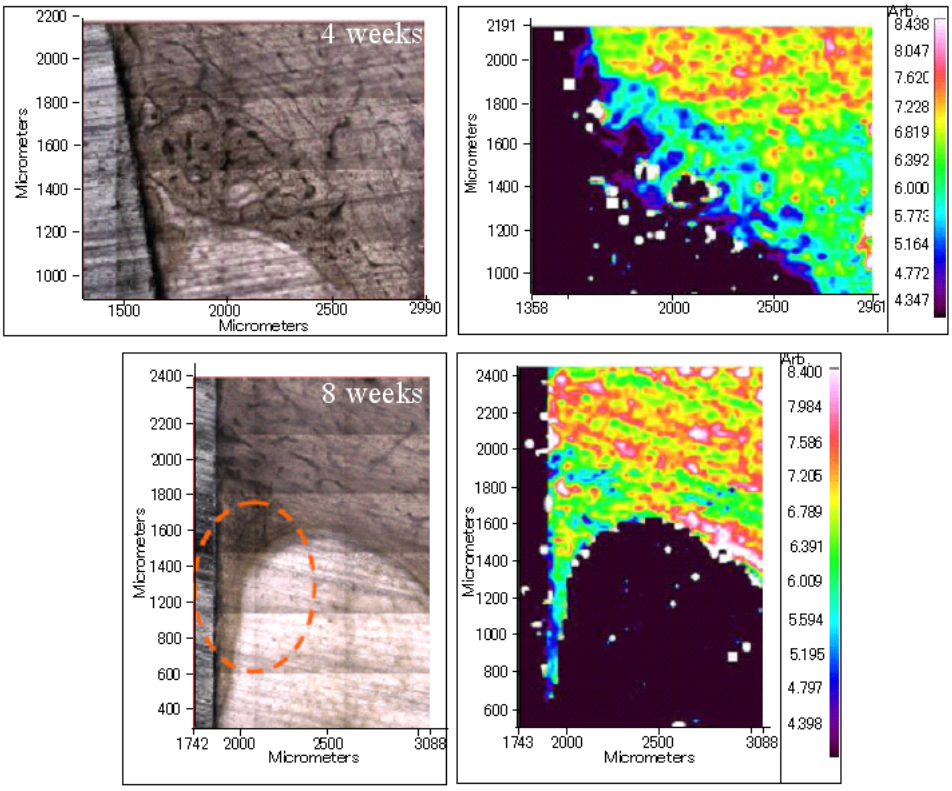

Fig. 6. PO_4^{3-}/Amide I ratio distribution in treated group-type 2 observed by FT-IR analysis.

under polarizing microscope examination was observed with extinction position as well as diagonal position alternately. In the extinction position, the collagen fiber lines up parallel to the direction of the observation and become darkness; similarly in the diagonal position, collagen fiber lines up vertical to the direction of the observation and becomes brightly. Therefore, we observed presence of haversian system in lamellae bone in the cortical bone of the samples.

Treated group

Bone was extended from the existing bone to the implant of the treated group at 4 weeks, the observation image of the cross polarizer method shows the irregular arrangement in the cord-like shaped in the extinction position and diagonal position, and the border of the existing bone was clear because lamellae structure is not formed. After 4 weeks of implantation, there was presence of new bone formation, which was still immature tissue. At 8 weeks, many layer of traverse lamella are parallel in the new bone, with evidence of tubular structure and haversian system, and the border with the cortical bone was unclear. But few lamella number was still observed, it was verified that the new bone at 8 weeks was still in felt condition lamella and in the stage of smallest lamella in the surface treatment group because it was insufficient also the regularity of travelling of collagen fiber in each lamella

Irregular orientation of collagen fiber bundles was seen in the new bone at 4 weeks as well as 8 weeks.

Control group

The amount of new bone formation was less in the control group compared with the treated group at 4 and 8 weeks. The collagen orientation was difficult to observe; and the maturity of the new tissue was detected almost at the same period of time of the treated group.

3. FT-IR analysis

In this experiment, the bone-implant interface of 2 kind of surface treatment (type 1 and 2) was evaluated. The figure 5 shows the interface of treated group-type 2 after 4 weeks of implantation by FT-IR analysis. The figure 5C and 5E demonstrated the existence of the main components of the bone: PO_4^{3-} (Hydroxyapatite origin), CO_3^{2-}(PO_4^{3-} inside of Hydroxyapatite, OH^- replaced by CO_3^{2-}), Amide I～III (bone is composed of proteins, mainly type I collagen). The infrared imaging obtained in the present research showed the same characteristic of bone tissue that was previously reported.

Treated group

A weak absorption of PO_4^{3-} and CO_3^{2-} were clearer observed in the cortical bone compared with the new bone at 4 weeks after implantation. Moreover, there was not a gradually migration of PO_4^{3-} and CO_3^{2-} from the cortical bone to the new bone, being the border line between the cortical bone and the new bone relatively clear. In addition, there was no difference between existing bone and new bone in the distribution of CO_3^{2-}／PO_4^{3-} ratio. The figure 6 shows the PO_4^{3-} ／ Amide I distribution ratio in treated group-type 2 at 4 and 8 weeks. PO43-/Amide I ratio in new bone was clearly lower than the existing bone at 4 weeks. Moreover, this component had lower value in new bone surrounding the implant than in the neighborhood of the existing bone. It was relatively clear the border between the new bone and the cortical bone at 4 weeks. At 8 weeks, it showed low value of PO_4^{3-}／Amide I ratio in the new bone compared with the cortical bone, especially the bone area contacting the implant. In summary, there was a higher amount of PO_4^{3-}／Amide I ratio in the new bone at 8 weeks than at 4 weeks. Moreover, the border between the new bone and the cortical bone at 8 weeks become unclear

Control group

The control group was excluded in this evaluation because there was not new bone formation.

Discussion

Recently, dental implants have gained much attention for a great number of researchers in dental application. Until now, there are few reports about the newly bone formed surrounding the implants, their properties and metabolism related to the bite force response, which are poorly understood. So far, the examination of the new bone was overlooked; because of this, the purpose of this research was to evaluate the bony tissue by Micro-CT, polarizing microscope and FT-IR analyses. The results observed in the present study shows that the difference of new and existing bone became clear in each technique. Furthermore, we could recognize the difference of newly bone formed at 4 and 8 weeks after implantation.

For the analysis of hard tissue, there are many samples in which are necessary the extraction of organic component as well as powdering. [15].The bone tissue consists of organic component (mainly collagen) and mineral component (microcrystal apatite) which are closely each other; because of this, when organic element is removed there is a risk of apatite denaturation. In addition, when the hydroxyapatite is powdered, the cristallinization is damaged decreasing the reliability of the result. Therefore, in this time, techniques which can collect data of the new bone without destructing the samples are required. Recent years, it has been developed and quickly diffused the Micro-CT analysis, which has taken a great attention because its results is from a three dimensional image of non-destructive bone tissue.

Moreover, it is possible to offers another kind of analysis of the samples because is not necessary any special pretreatment in the specimens. Thus, for this advantage, Micro-CT was used for observing the samples before performing any processes. The current result showed clearly large amount of new bone formation

attached to the existing bone in the control and treated groups. For that reason, we believe that osteoblast and precursor cells close to the existing bone have many supplied advantages surrounding the implant. [16]. Besides, in early stage at 4 weeks, the amount of the new bone formation was higher in the upper side of the implant than in the lower part. Thus could be due to the presence of granulation tissue with endothelial cells, fibroblasts and macrophage with the secretion of cytokines and growth factor in the upper side of the implant. We believe that these cytokines and growth factors act in an early stage inducing osteoblast differentiation and bone formation.

Therefore, surrounding the implant entrance, various cytokine and growth factor are secreted from vascular endothelial cells, fibroblast as well as from macrophage forming a granulation tissue; activation of cell occur concerning osteoblast as well as bone formation; we thought it is the region that can possibly grow the bone early result.

Nuzzo et al. reported that the changes of calcium deposition degree from woven to matured bone induced by drugs can be estimated by Micro-CT analysis. [17]. Moreover, It has been published that trabecular bone, cortical bone and intracortical porosity of mouse's femur in three dimension can be estimated[18]; there is also reported that, three dimensionally, vessel canal system in cortical bone of human femoral neck makes the quantification possible[19].

In the current study, the observation target is the new bone surrounding the implant, which could not obtain a high resolution being influenced for an artifact. It could not do micro observation of bone structure but it could confirm an increase of radiopacity of new bone in the treated group from 4 to 8 weeks.

All collected elements crossing the x-ray were expressed as radiopacity by Micro-CT analysis. Especially the Ca included in bone which is a heavy chemical element with high radiopacity; when increase of amount of Ca increases also the radiopacity, that's way it is possible to observe calcification and bone mineral amount as radiopacity by CT. Therefore, the CT image revealed higher calcification in the new bone at 8 weeks compared to 4 weeks. However, in this study, an evidence of artifact in the area of new bone surrounding the Ti alloy was detected affecting the results. We hope to get solution to reduce the artifact in both hard and soft tissues.

The polarizing microscope properties exhibit characteristic of polarized light image and interference image due to the double refraction in organic as well as inorganic component of hard tissue [13]. For that reason, we could easily observe the morphological information of bone tissue which is difficult to see from a common light microscope. Besides, Back Scattering Image (BSI) has the necessity to evaporate carbon in the samples while in polarizing microscope is no necessary to apply a pretreatment in samples in the existing bone and the new bone comparing with light microscope which is commonly difficult for estimating the bone tissue without staining, as well as it is possible to obtain information about the maturity stage of bone. Nakada et al. reported that is possible to obtain information about ontogeny stage of bone surrounding the implant using polarizing microscope[21]. In this study, we could clarify the difference of maturity level of new bone at 4 and 8 weeks according to the collagen fiber orientation inside the matrix of the new and existing bone. The new bone was still immature at 4 weeks, but in the new bone at 8 weeks partially mature bone was observed; there was part in which exhibit irregular layer structure and part in which exits together with scattered cord-like fiber, and the border of new and existing bone became unclear. This result indicates that we got rapid metabolic turnover in the new bone from 4 weeks to 8 weeks; we thought there was a maturity process. It has been published that it was observed bone tissue on the implant inserted in tibia of rabbits after 4 weeks of implantation by BSI; it is reported that it was possible to recognize the border of new bone and existing bone by different color tone. [22]. In this research, the treated group showed a clear border of new bone and existing bone from the orientation of collagen fibers at 4 weeks. At 8 weeks, the lamella structure in a part of new bone and the border with the existing bone became unclear, but it did not reach to the regular lamella structure as in the existing bone. Therefore, the new bone surrounding the implant did not get the maturity of the existing bone at 8 weeks. In the sensitive color plate method, because it can identify the intensity and orientation of the double refraction of collagen fiber due to color, the difference of structure of the existing bone and the new bone was observed more clearly. From the results observed above, in the future we expect to get maturation of formed bone similar to the existing bone after 8 weeks; we would like to do further researches.

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The infrared absorption spectrum obtained by FT-IR, especially in 2000-700cm^{-1} called fingerprints region, is capable to obtain information about chemical structure of sample because they are characteristic molecule[23]. There are many comparative studies of normal bone and bone disease by FT-IR analysis reported until now [24-29]; moreover, FT-IR is a reliable technique that make possible the analysis of bone tissue [30-31]. However, it is not published any case in which is observed chronological molecule

distribution of the new bone surrounding the implant. In this experiment using FT-IR devise, it was clearly detected the content ratio of molecules because of the difference in bone tissue of the existing and new bone.

Hydroxyapatite is an inorganic component of bone tissue, in which it is possible to know the amount of inorganic component of the sample from the distribution of PO_4^{3-} because has hydroxide, calcium and phosphate. In the same way as for organic component of bone tissue, mainly because it is collagen type I, it is possible to know the amount of inorganic component content in the sample because of the distribution of Amide I-III. This time, it was examined concerning Amide I whose absorption is stronger in comparison with Amide II and III. Calcification is the phenomenon in which detect the inorganic component of hydroxyapatite in bone [32]. Therefore, it is possible to know calcification of samples from PO_4^{3-}/Amide I ratio[14]. It is well known that apatite present in the body have crystal distortion, where chemical elements substitute various extremely small molecules like CO_3^{2-}, Na^+, Mg^{2+} in the position of Ca2+, PO_4^{3-}, OH- [9]. Especially the substitution ratio of CO_3^{2-} is high, that amount in bone tissue is reported approximately in 7%[8], that vary according on the analyst. For that reason, we believe that all apatite differ according to animals, age, sex, health and position, being difficult to verify clearly the apatite formation and growth mechanism. On the other hand, also there are reported researches in which examine the maturity of crystal from measurement of CO_3^{2-}/PO_4^{3-} ratio[14].

In this research, in the treated group at 4 weeks was clear the difference of distribution amount of PO_4^{3-}, CO_3^{2-} in existing bone and in new bone. The distribution amount of PO_4^{3-}, CO_3^{2-} in new bone was lower compared to the existing bone; the border of both bones was clear as well as the microscope finding. Therefore, it was thought that the state of inorganic accumulation, generally, is smaller in the part of the new bone compared with the existing bone at 4 weeks. Also, it could recognize a clear difference of PO_4^{3-}/Amide I ratio in the new bone and in the existing bone at 4 weeks. These results indicate that there was not enough calcification after 4 weeks of implantation in the new bone, but high calcification was detected in the existing bone. It could recognize also the difference in the new bone with the existing bone, in the treated group at 8 weeks, in PO_4^{3-}, CO_3^{2-} as well as PO_4^{3-}/Amide I ratio. It showed lower distribution in the new bone compared with the existing bone identically at 4 weeks.

But, because it became spread the part where PO_4^{3-}/Amide I ratio is high in the new bone close to the existing bone compared with 4 weeks, the border became a little unclear. It still showed a low value of PO_4^{3-}/Amide I ratio in the part of the new bone which is far from the existing bone. Therefore, it was thought that it is the process which is growing the matured bone tissue which advances the calcification gradually from the part close to the existing bone in the new bone at 8 weeks.

Burr et al. reported a decrease of CO_3^{2-}/PO_4^{3-} ratio measured by FT-IR, when microdamage occurs as a result of cycling loading placed on bone tissue[33]. Boskey et al. reported that CO_3^{2-}/PO_4^{3-} ratio of the bone raises with growth of tissue. In this study, we believe that calcification degree of the new bone has not reach the mineral maturity as the existing bone because CO_3^{2-}/PO_4^{3-} ratio was higher in existing bone compared with the new bone at 4 weeks.

In recent years, with the purpose of recover functionality as well as early esthetics, there were experimental studies of immediate loading of implant. In this study, at 4 week there was evidence of immature bone supporting the implant, which was clearly differentiated from the existing bone. On the other hand, most part of the mineral maturity of the new hard tissue was closely similar to the existing bone at 8 weeks. It is reported that remodeling bone is accelerated by the increase of dynamic stress and also internal constitution of the bone change by dynamic request[16]; the future goal of this study is the examination at the same time of bone tissue estimation with proper bite force timing in implant.

Until now, there are many studies focused in the measurement of hard tissue research; however, the mechanism of bone formation as well as the process of calcification is still unknown. Also, using one method of measurement, partial or non-information was obtained; because of this it was necessary to observe the bone from all sides using several method of measurement. However, in the usual analysis of scanning electron microscope or light microscope pretreatment as evaporation, ink spattering or staining are required[34-35], because of this the same sample is prepared with others methods to be analyzed. All analysis used in this study do not require any pretreatment of the sample before the measurement, thus the identical area of the same specimen can be observed with different analytical method , and is able to perform a comparative study with the obtained results; at the same time it could verify reproducibility.

This time it was estimated the new bone surrounding the implant; in Micro-CT it could observe three-dimensionally aspect like new bone formation in the surface of the implant. On the other hand, FT-IR analysis was weak for the detection of Ca content, while Micro-CT could confirm the difference of degree of calcification of existing bone and new bone. It could confirm the maturity of lamella structure of bone in polarizing microscope. It could confirm the difference of chemical structure of existing bone and new bone by FT-IR imaging measurement. So far, there were few reports about the qualitative difference between the existing bone and the new bone formed surrounding the implant, where both hard tissues have the same classification. However in this research, it recognize that there are difference in bone tissue of new bone and existing bone in the three used analysis; furthermore, it became clear the difference between new bone at 4 and 8 weeks.

Osseointegration plays an important role for an agreeable

clinical result of Ti-implant treatment

Recently, in the clinical trial, the success or failure of osseointegration depends of standard healing period, inspection of tooth mobility or x-ray inspection, age, sex, whole body condition, etc.

Because it is the request for the clinician, we would like to find out a solution for this problem; for that reason, we plan to study in a near future the detail of the bone formation process

Conclusions

The results of the current study analyzed by Micro-CT, polarizing microscope and FT-IR examinations indicated that:1) It was observed a qualitative difference in the new bone formation and the existing bone at 4 and 8 weeks of implantation; 2) There was also a qualitative difference in the newly bone formed at 4 and 8 weeks; 3) The maturation of the new bone started from the exhibiting bone; and 4) None of the methods used in current study destroyed the samples, and reproducibility results were obtained. In conclusion, we suggest a possible qualitative evaluation of new bone formation surroundings the Ti implant.

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