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Original

Observation of Newly Formed Bone Around Implants Using Parametric X-ray

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Abstract: Succesful dental implantation requires high osseointegration of the implant with the bone tissue that should continue for many years after implantation. Many studies on bone formation around implant have used histological evaluation, which is limited because it is qualitative. Even the information obtained using of back-scattered electron microscopic imaging are not sufficiently detailed. The purpose of this study is to determine if parametric X-ray radiation (PXR) could be used to obtain more quantitative information on newly formed bone around implants. Ti alloy implants grit-blasted with apatitic abrasive inserted in surgically created defects in rabbit tibia were retrieved after 2 weeks. Implant and tibia bone were imbedded in osteoresin, polished and sectioned (50µm) and examined using PXR.

Results showed that resolution of bone transmission images from PXR depended on the wavelength: wavelength set at 0.954A (13 KeV) did not show the newly formed bone around implants, but at wavelength set at 1.771A (7KeV) showed high resolution and clearly differentiated cortical bone from newly formed bone around implants.

This study demonstrated that hard tissue structure X-ray image observation with a high resolution was possible using PXR apparatuses, one of which was installed in the Laboratory for Electron Beam Research and Application (LEBRA) Institute of Quantum Science, Nihon University (LEBRA-PXR).

Key word: implant, parametric X-ray, rabbit, X-ray

Introduction

Successful dental implantation requires high osseointegration of the implant with the bone tissue for many years after implantation[1,2)]. The osteogenic processes in forming bone proceed in the order: fibrous bone, felt-like bone, minimum lamellar system à Haversian lamellar bone[3)]. However, the processes of bone formation around or associated with the titanium or titanium alloy implant is not well known. We previously reported that there were different structure types in newly formed bone around implants[4,5)].

The methods used for analyzing bone tissue around implants include: optical microscopic image analysis, contact micro-radiography (CMR), scanning electron microscopy (SEM), transmission electron microscopy (TEM), fourier transform infrared spectroscopy (FTIR), energy-dispersive X-ray spectroscopy (EDS), X-ray diffraction (XRD), auger electron spectroscopy, and X-ray photoelectron spectroscopy (XPS)[6-10)].

Parametric X-ray radiation (PXR) apparatuses, one of which was installed in the Laboratory for Electron Beam Research and Application (LEBRA) Institute of Quantum Science, Nihon University (LEBRA-PXR), are the first constantly available PXR generation apparatuses[11)] in the world, featuring arbitrary wavelength establishment. The PXR is generated by using electron and crystal interaction producing very bright and intense radiation (Fig1). This allows non-destructive analysis and rapid analysis. Image resolution is improved by the ability to select optimum wavelength.

However, even by these methods, detailed analysis of the new bone formation around the implant is difficult.

The purpose of this study is to determine the potential of The LEBRA-PXR imaging and conventional dental X-ray imaging in investigating the details of newly formed bone around the implants.

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Materials and Methods

1. Test materials

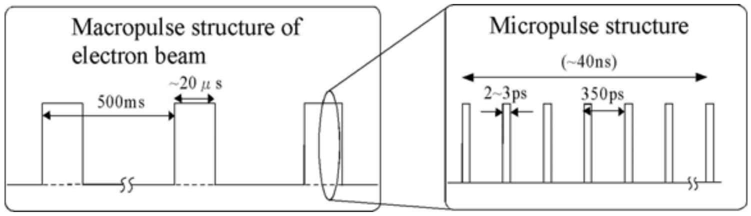

Fig.1 The structure of the electron beam pulse of the LEBRA-LINAC. The macropulse separation was 500ms and the pulse width was 20ìs at 2Hz. When a pulse was magnified, the separation was 350 ps with microp

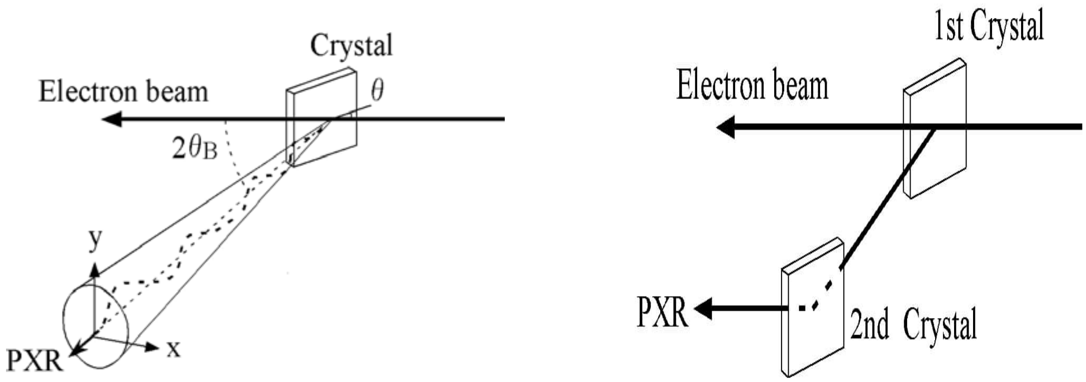

Fig. 2 Schematic diagram of the PXR generator. The electron beam from the LINAC accelerator generated PXR from the bombardment of the first crystal, and a cone-shaped electron beam radiated with a divergence angle of 46ìrad (x-axis direction) and 240ìrad (y-axis direction). The second crystal, which was placed parallel to the first crystal, diffracted the PXR. The setup was established so that the PXR output was constantly in one direction.

The materials were titanium alloy implants (2.8mm in diameter and 8 mm in height) grit-blasted with apatitic abrasive (MCD, HiMed, Bethpage,Long Island).

2. Implantation methods

Experimental animals were 13-week-old (2.5 kg weight) New Zealand White Rabbits (Sankyo Lab Service Co., Japan) The Nihon University School of Dentistry at Matsudo Experimental animal Ethics Committee (ECA-03-0003) approved the experimental protocol for the use of the animals. The Ti alloy rods were implanted in surgically created holes (2.8 mm) in rabbit tibias and the holes without implant served as controls. The rabbits were sacrificed under anesthesia after 2 weeks after implantation (n=3). Non-decalcified histological sections (50μm) were obtained from the implant sections in the trabecular bone areas. Implantation methods and preparation of specimens for analysis are described in the references[4,5].

3. Observation of X-ray transmission images by LEBRA-PXR

Observation of X-ray transmission images were made using the PXR equipment installed in the LEBRA, Institute of Quantum Science, at Nihon University. The LEBRA-PXR is dedicated to a wide-range studies using PXR. X-ray of the PXR is generated by irradiating crystals with high-speed electron beams released from these accelerators, and the LEBRA-PXR generation apparatus contains a 125 MeV linear electron accelerator for the basic and applied studies in accelerator science, in which the PXR is generated by driving super-short pulses of electron beam accelerated by a linear accelerator (LINAC) (Fig. 1) into crystals (Si). The PXR is generated in a constant direction parallel to the electron beam direction by arranging 2 crystals, and the X-ray generated has excellent characteristics such as high brightness, ultra-super short pulses, and variable wavelength (Fig. 2).

Using non-decalcified specimens and the PXR generation apparatus, which is a continuous wavelength variable monochrome X-ray beam source developed in the LEBRA, the PXR observation was performed under the following measurement conditions: Electron linear accelerator parameters: electron energy 100MeV, macro-pulse width 20μm, macro-pulse current 90mA, repetition frequency 2Hz, and mean beam current 3.6mA. Target (used as a PXR generation source): silicon single crystal (111) surface, X-ray energy 7KeV (1.771Å) and 13 KeV(0.954Å), exposure duration 900s (20μs×1,800 shots and actual irradiation time 36ms), distance between the beam source and irradiation site: approximately7.5m, apparatus used for imaging: imaging plate (IP), and IP reading apparatus: YCR-21XG® (Yoshida Factory Co.) (reading resolution 29μm×29μm). Details of LEBRA-PXR are described in the references[11].

Measurement conditions of a conventional dental X-ray apparatus (SIEMENS Co., Heliodent MD) were X-ray energy

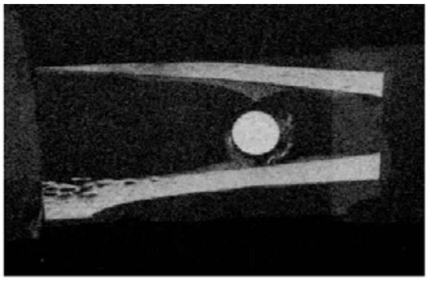

Fig. 3 Image of newly formed bone around implant taken by LEBRA-PXR imaging (7keV, 1.771Å).The implant was observed to be radiopaque with 2.8mm diameter. A layer of new bone was observed, one portion of which was observed to be calcified much like the surrounding cortical bone.

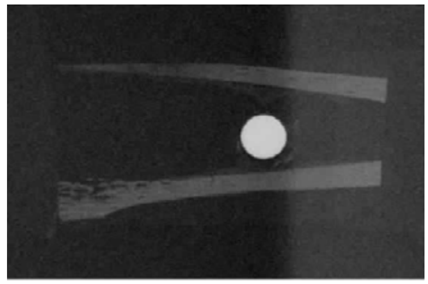

Fig. 4 Image of newly formed bone around implant taken by LEBRA-PXR imaging (13keV, 0.954Å).The implant was observed to be radiopaque with 2.8 mm diameter. The cortical bone in the implant surrounding was observed, but the new bone appeared blurred and indistinct in the image.

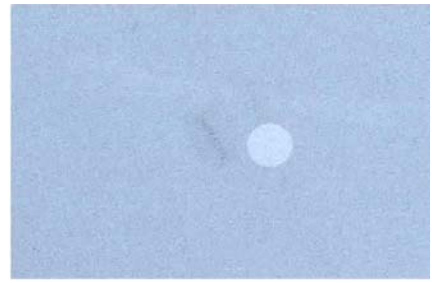

Fig.5 Image of newly formed bone around implant taken by a dental x-ray machine.The implant was observed to be radiopaque with 2.8mm diameter. The new bone as well as the cortical bone surrounding the implant were observed to be indistinct and radiopaque. The discrimination of structures was almost impossible in the image.

60KeV, exposure duration 0.25s, distance between the beam source and irradiation site: 0.5m.

Results

High-contrast transmission images were obtained at wavelengths set at 7KeV, 1.771Å (Fig. 3) and 13KeV, 0.954Å (Fig. 4) (EkeV = 12.40/lÅ). At the wavelength of 0.954Å(13 KeV) (Fig. 4), although substance transmission was detected at this short PXR wavelength, contrast was poor and unclear transmission images were found around implants. At the wavelength of 1.771Å (Fig. 3), since it is longer than 0.954Å(Fig. 4), substance transmission should become weak; however, the specimens in this study showed a better contrast, and the degree of calcification of new bone formed around implants was clearly noted. Fig. 5 shows X-ray images obtained by conventional dental X-ray. Although various conditions were set in order to obtain contrast as good as that at the PXR wavelength of 1.771Å (Fig. 4), images of not only new bone but also cortical bone were unclear, showing almost monotonous X-ray transmission images.

Discussion

It is well known regarding X-ray transmission image observation that observation images can be made clear and quantitative measurement is possible by changing X-ray wavelengths according to the components of the subject substance. Since X-ray transmission image observation of substances is advantageous to the investigation of crystal structures including body crystal substances, and functions elucidated from the investigation results, it has been applied to medical diagnoses to obtain basic information regarding pathologic disease conditions[12,13)]. In particular, by changing X-ray wavelengths, transmission images related to substance components are obtained, and measurement of bone density and bone calcification degrees is possible for medical diagnosis[14,15)]. The roentgen photographic images obtained by the use of a dental X-ray apparatus did not differentiate bone changes and were unclear except for showing implants, due to almost monotonous transmission of X-ray influenced by the specimen thickness, which was approximately 50μm. Recently, digital X-ray has been used clinically, and high-resolution images are expected; however, since the resolution of the dental X-ray used in this study was lower that that of digital X-ray, detailed observation of the specimen structures on a microscopic level was impossible. By transmission image observation of the same specimen at the wavelength of 1.771Å using the LEBRA-PXR, cortical bone was clearly observed, and the formation of immaturely calcified newly formed bone around implants was confirmed. On the other hand, using the same LEBRA-PXR apparatus, when the wavelength was set to 0.954Å, clearness of images decreased compared with that at 1.771Å. This was considered due to differences in substance transmission energy between the 2 wavelengths.

Therefore, our results revealed the advantage of the LEBRA-PXR with variable X-ray wavelength in X-ray transmission image observation.

Although the theory and application of the PXR have been studied for many years, there are a few institutions performing experimental studies in the world[16-19)], and no institution provides the use of PXR for actual studies. Other than super-large light-radiation institutes such as Spring-8 and KEK, it was difficult to change X-ray wavelengths. Furthermore, in cases of tube type X-ray generation apparatuses, although X-ray wavelength can be changed by selecting the type of the cathode, since the effects are due to differences in atomic numbers, the output X-ray wavelength changes in stages; therefore, it is impossible to select arbitrary X-ray wavelengths. However, changing wavelengths arbitrarily by the PXR method using a small LINAC in this study, we revealed that higher resolution images of hard tissue structures were obtained by LEBRA-PXR, compared with those in hard tissue structure analysis using conventional apparatuses. It is considered that hard tissue structure analyses and elucidation of formation mechanism will greatly advance using the LEBRA-PXR for hard tissue research.

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