Potential Application of High-Resolution Microfocus X-ray Techniques for Observation of Bone Structure and Bone-Implant Interface

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Purpose: *The aim of the present study was to evaluate the potential application of 2 types of microfocus x-ray units to study the bone structure around dental implants and at the bone-implant interface.* ***Materials and Methods:*** *IMZ titanium implants were placed in the maxilla and mandible of a beagle dog. After implantation periods of 1, 2, and 3 months, the bone-implant interface was evaluated with microfocus x-ray computed tomography (CT) and microfocus x-ray fluoroscopy.* ***Results:*** *Microfocus x-ray CT images of the bone-implant specimen at 3 months after implant placement revealed a clear distinction between the implant and the bone. The implant surface was partially covered with bone, and direct contact between the implant and bone could be clearly seen. Differences in degrees of calcification were identified by the differences in relative black and white intensity. Microfocus x-ray fluoroscopy also showed clear features of the bone and titanium implant. The original drill hole and new bone formation could be recognized. These findings corresponded with traditional histologic observations by light microscopy.* ***Discussion and Conclusion:*** *Microfocus x-ray techniques are non-destructive and require a very short examination time. They are considered useful to observe details of the bone structure and bone-implant interface. Microfocus x-ray fluoroscope and microfocus x-ray CT techniques can provide a clear and distinguishable image of the bone-implant interface because of their high spatial resolution.* (INT J ORAL MAXILLOFAC IMPLANTS 2003;18:279–285)

Key words: *bone structure, dental implants, dental radiography, microfocus x-ray*

The success of dental implants may be attributed to the osseointegration between the implant material and host bone.[1–4] Conventionally, undecalcified thin sections are an important factor in demonstrating osseointegration. The preparation of thin sections is a very laborious and time-consuming procedure that requires special knowledge and skill. Furthermore, artificial fissures sometimes occur at the bone-implant interface, which may lead to misinterpretation.

Radiography and computed tomography (CT) are expected to be improved techniques for studying bone structure or the bone-implant interface, because they provide sectional images of internal structures without tissue sections and without destroying the specimen. CT is now widely used for the diagnosis of diseases in dental and medical fields. However, it is difficult to observe and analyze fine structures less than a few cubic millimeters in size because of its low spatial resolution, which is more than 100 µm. Conventional radiographic techniques do not provide high enough magnification or spatial resolution for the characterization of bone and the bone-implant interface.

Bonse and coworkers[5] studied absorption-contrast microtomography using synchrotron radiation

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and achieved a spatial resolution of 8 µm for human cancellous bone. Momose and associates[6] observed the soft tissue of cancerous rabbit liver by a modified phase-contrast technique using synchrotron radiation with a spatial resolution of 30 µm. However, the equipment for synchrotron radiation takes up a great deal of space and is expensive to operate.

A new type of microfocus x-ray unit with a spatial resolution less than 10 µm has been developed (Nittetsu Elex, Kanagawa, Japan; Shimadzu, Kyoto, Japan). A magnetic focus-control microfocus x-ray tube was used as an open-vacuum x-ray source, which produces a fine and equal-speed electron beam. Shibata and Nagano[7] applied this type of microfocus x-ray CT to observe the auditory ossicles of a human fetus, the smallest and lightest bones in the skeletal system. The high-resolution microfocus x-ray technique has been used effectively to study trabecular bone structure,[8,9] enamel thickness,[10] and calcification of human teeth,[11] as well as dental root canal morphology.[12,13]

A few studies have examined the bone-implant interface or bone structure around implants using microfocus CT. Sakae and colleagues[13] were able to observe the compact and spongy bone architecture clearly on microfocus tomograms, but noted artificial concentric circles related to the difference in x-ray absorption coefficient between bone and the titanium implant. Van Oosterwyck and coworkers[14] validated the qualitative accuracy of microfocus x-ray CT images by comparing histologic sections with the corresponding CT slices for the same specimen. Their microfocus x-ray tube had a focal spot of 10 µm.

The present study examined a recently developed microfocus x-ray machine that uses an x-ray tube with a focal dimension of 4 µm. The aim was to explore the potential application of 2 types of microfocus x-ray machines—the microfocus x-ray fluoroscope and the microfocus x-ray CT system—to study the bone structure around implants and the bone-implant interface by comparing the images produced by these techniques with stained histologic sections and contact microradiograms.

MATERIALS AND METHODS

Animal Model and Surgical Procedures

The Animal Care and Use Committee of the College of Bioresource Sciences, Nihon University, Japan, approved the protocols and guidelines of this study. A female beagle dog weighing 11 kg was used in the study. The maxillary and mandibular premolars were extracted to create edentulous regions in the jaws. Healing of alveolar bone was achieved approximately 1 year after extraction.

At that time, titanium spray-coated IMZ implants (Friatec AG, Mannheim, Germany) 3.3 mm in diameter and 10 mm in length were placed. The basic IMZ implantation procedure was performed according to the manufacturer's instruction. All surgical procedures for implantation were performed under general anesthesia. Atropine sulfate (Tanabe Seiyaku, Osaka, Japan) 0.04 mg/kg was administered subcutaneously, followed by intravenous sodium thiopental (Tanabe Seiyaku) 17.5 mg/kg. Then 2% isoflurane (Dainippon Pharmaceutical, Osaka, Japan) was given continuously through an 8-mm tracheal tube at an oxygen flow rate of 2 L/min as inhalation anesthesia.

First Implant Surgery. First, 2 implants were placed in the mandible. Crestal incisions were made bilaterally in the mandible, extending from the canine to the first molar. Full-thickness mucoperiosteal flaps were reflected. A small, sharp periosteal elevator was used to separate the periosteum. After exposing the mandible in bilateral premolar regions, pilot holes of 0.6 mm were drilled. The holes were gradually widened with successive drills up to the final diameter (3.3 mm) of the implants. The bone preparation was performed with very gentle surgical techniques using a low drilling speed (500 rpm) and continuous internal cooling with a bone surgery system (Royal, Tokyo, Japan). After press-fit placement of the implants, the soft tissues were closed in separate layers using intracutaneous resorbable Vicryl 3-0 sutures.

Second and Third Implant Surgeries. The second implant placement surgery was performed in the maxilla 1 month after the first implantation. The premolar region of the maxilla was exposed in 1 quadrant, and 1 IMZ implant was placed using the same method as described above. The third implant surgery was performed 1 month after the second. One IMZ implant was placed into the remaining maxillary premolar region with the same surgical procedure.

The dog was fed a soft diet after the implant surgery. To reduce the risk of postoperative infection, prophylactic antibiotic enrofloxacin (Baytril; Bayer AG, Leverkusen, Germany) was administered preoperatively by subcutaneous injection. The position and stability of the placed implants during the healing period was examined by a dental x-ray unit (Max-F1-M, Morita, Kyoto, Japan). All implants demonstrated good primary stability. The health condition of the dog was always checked before and after the surgery, and there were no signs of infection. The weight of the dog remained constant during the experimental period.

Three months after the first implant was placed, the animal was sacrificed by injection of an overdose of pentobarbital sodium (Nembutal; Abbott Laboratories, North Chicago, IL). Three kinds of bone-implant specimens, ie, 1-month (1 implant), 2-month (1 implant), and 3-month (2 implants), were therefore obtained.

Microfocus X-ray Observations

After the animal was sacrificed, the maxilla and mandible were excised. The bone/implant specimens were fixed in 10% buffered formalin, dehydrated through a graded series of ethanols, and embedded in polyester resin (Rigolac; Nisshin EM, Tokyo, Japan).

The mandibular bone/implant specimen obtained 3 months after implantation was examined first, using a microfocus x-ray CT machine (SMX-225CT; Shimadzu, Kyoto, Japan). The microfocus x-ray source of the machine had a focal spot of 4 µm, maximum voltage of 225 kV, and maximum current of 1 mA. The x-ray generator and detectors (image intensifier) were stationary and the sample was rotated on stage. To obtain a cross-sectional image, the CT scanner system accumulated fluoroscopic images from all angles, and a computer reconstructed the inner structural image. The rotational stage moved perpendicularly; thus it was possible to obtain a cross-sectional image of the sample in any position desired. The minimum reconstruction time was 12 seconds for a 512×512 2-dimensional image matrix with an 1,800-angle view using exclusive high-speed computation hardware such as the Digital Signal Processor (NEC Electronics, Kanagawa, Japan). The digitized signals were further transferred to a personal computer equipped with Windows MNT 4.0. Reconstructed images were saved in .bmp or .jpg format.

Buccolingual tomograms parallel to the long axis of the implant were obtained starting from the center of the implant and moving sequentially outward at a slice thickness of 0.1 mm and slice pitch 0.8 mm, with the machine operated at a tube focal length of 4 µm, a tube voltage of 65 kV, and a tube current of 70 µA. The pixel number was 512×512.

Subsequently, the other specimens were studied in a similar way.

Next, 3-mm-thick buccolingual sections including the implant were prepared from the 1-, 2-, and 3-month specimens using a low-speed rotating blade microtome (Isomet; Buehler, Lake Bluff, IL). The bone structures around the implant were observed using a microfocus x-ray fluoroscope (SMX-80; Shimadzu) operating at a tube focal length of 4 µm, an anode voltage of 80 kV, and a tube current of 200 µA. Control of the x-ray source and image tube, positioning of the XY table, and image processing were possible using a personal computer with the Windows system. An AC servo motor was used to activate the XY table. Image-processing software (X-ray Image Viewer; Shimadzu) was incorporated. The fluoroscopic image was inverted and the negative image was used for observations.

Fig 1 Conventional dental radiograph of the implant and surrounding bone in a specimen obtained 1 month after implant placement. There is no distinct bone resorption at the implant shoulder. Ultra-speed film (DF-55, Eastman Kodak, Rochester, NY); X-ray source: 60 kV and 20 mA.

Histologic Procedures

For comparison with microfocus x-ray observations, undecalcified ground sections were prepared from the 1-, 2-, and 3-month specimens. After fixation, the specimens were dehydrated through a graded series of ethanols and embedded in polyester resin (Rigolac; Nishin EM). After polymerization, 100-µm-thick ground sections were prepared in a direction parallel to the axis of the implant, using a grinding stone. These histologic sections were first studied by a contact microradiographic technique and then stained with methylene blue and basic fuchsin. Contact microradiography was performed in an x-ray cabinet (SRO-M40; Sofron, Soken, Japan) using a high-resolution film (SO-343; Kodak, Rochester, NY) at 30 kV and 3 mA. The implant-bone interface was observed under a light microscope (Eclipse E800M; Nikon, Tokyo, Japan).

RESULTS

All implants were all clinically stable without any mobility. The gingiva around the implants showed no signs of inflammation. In the dental radiograph of a specimen obtained 1 month after implant

Figs 2a to 2c *Microfocus x-ray CT images of a bone/implant specimen obtained 3 months after implant placement.*

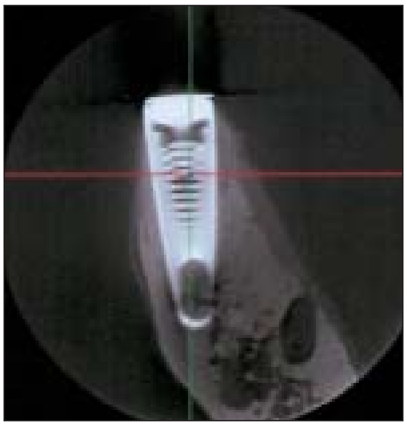


Fig 2a Original longitudinal CT image of a slice at the buccolingual region. The implant is seen as a white object that is clearly distinguishable from the bone, which appears gray. The difference in degree of calcification is identified by the difference in relative black and white intensity. The implant and the bone are in close contact. Slice thickness 100 µm; x-ray source accelerating voltage 65 kV; current 70 µA; geometric magnification ×5.97; spatial resolution 4 µm.

Fig 2b Computer-reconstructed image from the CT image of Fig 2a, on the mesiodistal longitudinal plane along the green line. Slice thickness 100 µm; x-ray source accelerating voltage 65 kV; current 70 µA; geometric magnification ×5.97; spatial resolution 4 µm.

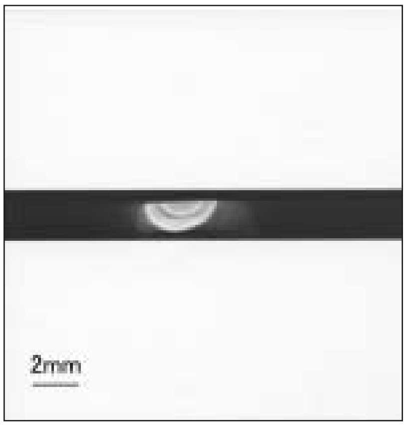


Fig 2c Computer-reconstructed image from the CT image of Fig 2a, on the transverse plane along the red line. Slice thickness 100 µm; x-ray source accelerating voltage 65 kV; current 70 µA; geometric magnification ×5.97; spatial resolution 4 µm.

placement (Fig 1), the bone around the implant showed no significant resorption.

Findings in Microfocus X-ray CT Images

Figure 2a shows a 2-dimensional, buccolingual CT of the 3-month bone/implant specimen. The CT data collection time was less than 60 seconds, and CT image reconstruction time was approximately 10 seconds. There was some evidence of resorption near the top of the alveolar bone on the buccal side, which could not be identified on conventional dental radiographs. The implant and the bone could be clearly distinguished. The implant was white and the bone was gray. The implant surface was partially covered with bone, and direct contact between the implant and bone could be clearly seen. The soft tissues covering the bone could also be clearly distinguished.

Figures 2b and 2c show the reconstructed CT of Fig 2a at the mesiodistal longitudinal plane (along the green line) and transverse plane (red line), respectively. The bone and implant could be seen clearly, even in the longitudinal and transverse reconstructed images, which could be obtained at any level of the implant by reconstruction of the integrated 2-dimensional CT slices.

Findings on Microfocus X-ray Fluoroscopic Images

One Month After Implant Placement. Compact bone could be seen in very close contact with the coronal part of the implant (Fig 3). There was new bone on the buccal side at the base of the implant, but no bone formation was evident on the palatal side.

Two Months After Implant Placement. Denser bone was evident around the implant in the cortical bone area. Fine trabecular bone of 100 µm in diameter could be easily identified. The original drill hole could be recognized (Fig 4, *arrowheads*), and new bone formation was observed between the original bone wall and the titanium implant.

Three Months After Implant Placement. Mature bone was observed around the implant (Fig 5). Direct contact between bone and implant was identified in some areas. An area of calcified material with uniform structure, which was subsequently identified as a remnant of the dental root tip, could be seen at the bottom of the alveolar bone on the lingual side of the implant (Fig 5, *asterisk*). The size of the root remnant was about 0.9×2.5 mm.

Microscopic Findings in Undecalcified Ground Tissue Sections and Contact Microradiograms

Figure 6 shows the light microscopic photograph of an undecalcified ground section at 2 months after

Fig 3 Microfocus x-ray fluoroscopic image of the maxillary premolar area implant 1 month after implant placement. New bone formation can be seen on the buccal side at the coronal part of the implant, but no bone formation is apparent on the palatal side. X-ray source accelerating voltage 80 kV; current 200 μA; geometric magnification ×1.0; spatial resolution 4 μm.

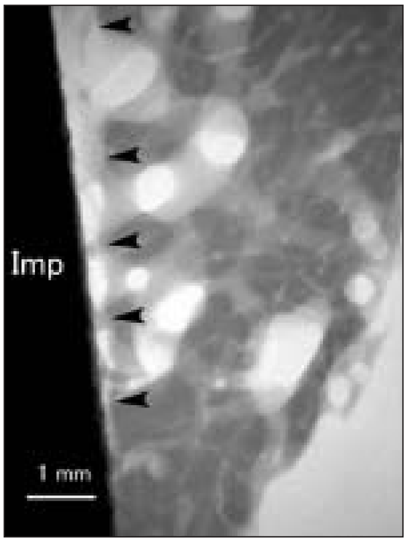


Fig 4 Microfocus x-ray fluoroscopic image of the maxillary premolar area implant at the highest magnification, 2 months after implant placement. New bone formation can be seen between the original bone wall *(arrowheads)* and the titanium implant. X-ray source accelerating voltage 80 kV; current 200 μA; geometric magnification ×1.0; spatial resolution 4 μm.

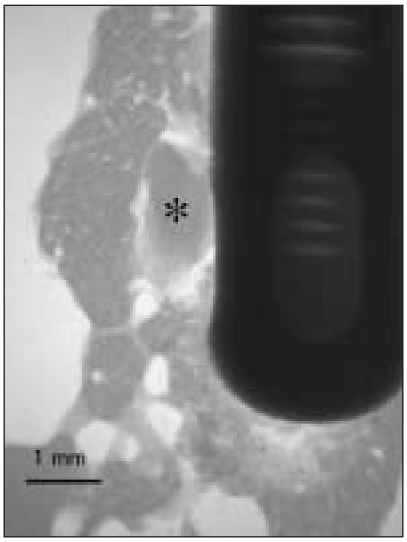


Fig 5 Microfocus x-ray fluoroscopic image of the mandibular premolar area implant 3 months after implant placement. Some direct contact between the bone and the implant can be seen. Calcified material with uniform structure can be seen at the lower part of the alveolar bone on the lingual side of the implant *(asterisk)*. This material was identified as the tip of the dental root. X-ray source accelerating voltage 80 kV; current 200 μA; geometric magnification ×1.0; spatial resolution 4 μm.

implant placement, corresponding with the fluoroscopic image of Fig 4. The implant was shown to have perforated the maxillary sinus at the time of implant placement (Fig 6, *arrowhead*). Mature bone surrounded the implant, and some direct contact between bone and implant could be seen.

Figures 7a and 7b show the light microscopic photograph and the contact microradiogram, respectively, of an undecalcified ground section at 3 months after implant placement. The substance with a uniform structure observed in the fluoroscopic image of Fig 5 was shown to have dental tubules, dental cementum, and dental pulp (Figs 7a and 7b, *asterisks*). Therefore, this appeared to be confirmation of a piece of remnant dental root left over from the time of tooth extraction.

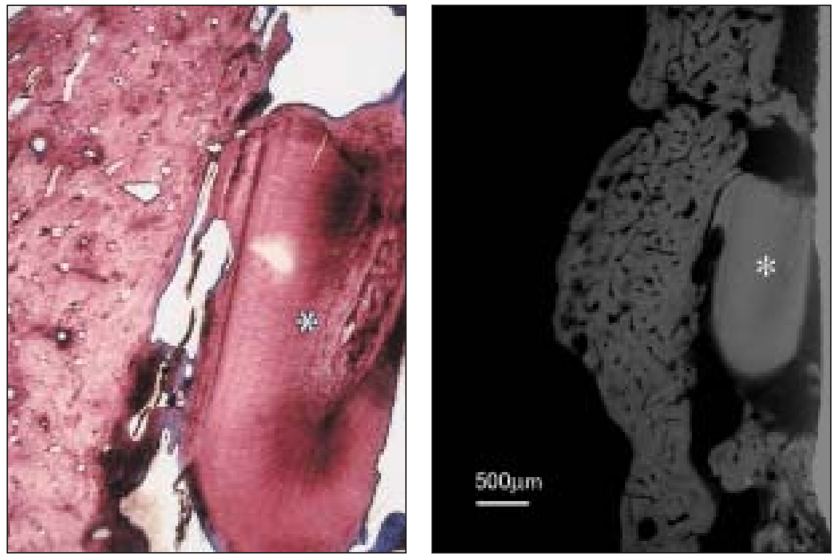
DISCUSSION

In the present study, bone formation and bone-implant contact were seen using the recently developed microfocus x-ray fluoroscopic and microfocus x-ray CT techniques. Light microscopic observations of undecalcified ground sections provided information on the state of bone formation and bone resorption around the implant and changes in soft tissues such as the gingival epithelium and subepithelial connective tissues.

Light microscopy can be an important tool for observing perforations of the maxillary antrum and pathologic changes in soft tissue such as gingivitis and maxillary sinusitis. The extent of calcification and the detailed structure of the hard tissue around the implant can be observed in a contact microradiogram of the ground sections. However, preparation of ground tissue sections is laborious and time consuming, and tissues are sometimes destroyed.

Observation with microfocus radiography is nondestructive and minimizes the risk of artifacts from sample preparation. Moreover, the observation can be completed within a very short time. The traditional dental radiograph identifies the position of the implant, and microfocus x-ray CT can be used to detect dental or medical conditions. However, both conventional techniques do not have high enough resolution to give any information about the detailed structure of the bone and/or bone-implant interface. Recently, microfocus x-ray equipment has been developed using the microfocus x-ray tube as a new generator. The microfocus x-ray tube requires a fine electron beam at constant

Fig 6 Light microscopic observation of an undecalcified ground tissue section 2 months after implantation. The implant perforates the mucosa of the maxillary sinus *(arrowhead).* Partial detachment between the bone and implant is observed. Mature bone is observed surrounding the implant, and some direct bone-implant contact is observed (methylene blue and basic fuchsin; original magnification ×20).



Figs 7a and 7b *(Left)* Light microscopic observation and *(right)* contact microradiogram of undecalcified ground tissue section 3 months after implant placement. The contact microradiograph was obtained using high-resolution film at 30 kV and 3 mA. The material with uniform structure observed in the x-ray fluoroscopic image has dental tubules, dental cementum, and dental pulp *(asterisk).* Therefore, it is apparently a remnant of dental root left from the time of tooth extraction (*Left:* methylene blue and basic fuchsin; original magnification ×40; *right:* original magnification ×20).

speed. This apparatus allows acquisition of radiographs within a short time and with high resolution. Clear observation of auditory bone structures, detailed trabecular bone structures, and teeth are possible using the newly developed microfocus x-ray technique.[7–13]

In the present study, it was possible to identify some resorption of the bone in microfocus x-ray fluoroscope images but not on conventional dental radiographs. This is the result of improvement of spatial resolution. It was also possible to distinguish the bone and the implant at high contrast in microfocus x-ray images and to identify direct contact between the bone and the implant, the very fine trabecular structure of newly formed bone, and the resorption of alveolar bone. There were no artificial concentric circles, which have been previously reported.[13] This was because of the development of the microfocus x-ray tube and/or image processing technique. The tube used in the present study has a focal dimension of 4 µm.

A piece of remnant dental root close to the implant could also be seen by microfocus x-ray technique, but histology was needed for identification. The time required for CT data collection was less than 60 seconds, and the time for reconstructing CT image was about 10 seconds. Thus, the microfocus x-ray technique can be very effective for making detailed observations of the bone structure around the implant and can save observation time. In the present study, formalin-fixed, resin-embedded samples were observed by microfocus x-ray. Although sample preparation did not cause serious deterioration of the reconstructed images, living tissues, if available, are preferred for observation.

Another advantage of microfocus x-ray technique is the ease of reconstruction of 3-dimensional (3D) images. Wigianto and associates[15] placed implants in rabbit tibiae and made 3D observations of the spongy bone structure around the implant. They embedded the bone containing the implant in resin, ground the sample sequentially from one side, and made observations of serial sections under a light microscope. These images were fed sequentially into a computer through a charge-coupled distributor camera and finally constructed into a 3D image. This method required considerable time and effort for specimen preparation. Van Oosterwyck and coworkers[14] produced 3D reconstruction images of bone/implant systems to create finite element models. They claimed that microfocus x-ray CT would be a powerful tool for the analysis of biomechanics using the finite element method. Reconstruction of 3D images of bone/implant specimens is now a matter of ongoing research.

Further evaluation of quantitative analysis using the microfocus x-ray technique is needed. The cost of the microfocus x-ray machine is still high for clinical usage. Tuned-aperture CT (TACT) is also a useful method to reconstruct 3D images from multiple 2D projections.[16,17] TACT is an accurate method that facilitates non-destructive assessment of bone or tooth structure, and it is cost-effective. The advantage of microfocus x-ray technique is its high spatial resolution. A comparison of microfocus x-ray technique and TACT for 3D image reconstruction should be performed.

SUMMARY

The results of this investigation indicate that microfocus x-ray techniques such as fluoroscopy and CT, which are non-destructive and involve a very short image acquisition time, can be advantageous tools for observing not only hard tissues such as bone and teeth, but also the interface between bone and titanium implants. Microfocus x-ray techniques can be expected to play an important role in medicine and dentistry because they allow visualization of the fine structures of hard tissues.

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