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Original

The Effect of Different Surgical Instruments for Bone Regeneration under the Surgery of Bone Defect on Rat Calvaria

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Abstract: In this study, we compared the healing process of bone defects treated with a trephine bur with those treated with an ultrasonic knife using a critical-sized bone defect model on rat calvaria. Nine-millimeter critical-size bone defects were prepared using both instruments in the calvaria of adult Sprague-Dawley rats. One and four weeks after the osteotomy, we performed a histomorphometric analysis to evaluate bone regeneration around the cutting surface. Quantitative micro-computed tomography analyses of the bone volume in both groups suggested that ultrasonic knife surgery resulted in superior bone formation compared to that in trephine bur surgery. Furthermore, at the cutting surface, the ultrasonic knife treatment retained the alkaline phosphatase activity and new bone formation, which was identified using calcein staining, even one week after surgery. Considering the speed and volume of bone regeneration, the ultrasonic knife is likely to be the preferred over the trephine bur to perform osteotomies in implant surgery.

Key words: Bone regeneration, Surgical instruments, Calvaria, Ultrasonic knife, Trephine bur

Introduction

In dental implantology, osteoblast attachment in the early phase is necessary, as it significantly influences implant fixation and is associated with implant survival[1)]. The placement of a dental implant in the bone activates a sequence of molecular and cellular events that lead to the apposition of newly formed bone directly onto the titanium surface[2)]. The surface structure of the implanted material and the condition of the surface of the implant bed both affect osteointegration[3)]. Dental implant failures that occur clinically for unknown reasons could be attributed to undiagnosed damage to the bone surface of the implant bed[4)]. Conventional rotary burs are frequently used for preparing implant drilled holes. Major problems encountered during bone drilling are thermal necrosis, bur deformation, and microcrack generation on the inner surface of the drilled holes, which can detrimentally affect the subsequent healing process[5)].

Piezosurgery using ultrasonic vibrations has been used for osteotomy since the 1950s as an alternative technique for surgery using a rotary bur[6,7)]. Ultrasonic instruments were used for implant surgery because they reduce the incidence of bone burns, and because these instruments could selectively cut the mineralized tissue[4)]. Surgery using ultrasonic instruments is known to reduce soft tissue damage because of the selective cutting that can be performed on mineralized tissue when the instruments are used at 25–30 kHz[8-10)]. Clinical studies have shown that ultrasonic techniques can provide a clear view of the surgical field, leading to reduced damage to essential anatomic structures such as the nerves or blood vessels[11,12)], reduced damage to the tissue wound leads to faster healing[13)]. In contrast, the conventional method that uses rotary drills in preparing implant sites causes thermal damage to the tissue in the implant bed, which may lead to reduced fixation and worse osteointegration around the dental implant[14)]. However, the differences in the bone regeneration process after osteotomy surgery using an ultrasonic knife or conventional rotary bur at the same surgical time remain controversial.

This study aimed to compare the effects of an ultrasonic knife and a trephine bur on bone regeneration used for similar durations on a rat critical-size defect model.

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Materials and Methods

Rat calvarial-defect model

Sixteen male Sprague-Dawley rats (8 weeks old, 250–270 g, SHIMIZU Laboratory Supplies Co, Ltd., Kyoto, Japan) were used for the animal studies. The experimental protocol was approved by the Animal Care and Use Committee of Osaka Dental University (approval number: 17-03008). The rats were divided into the following groups: (i) the trephine bur group, further divided into the 1- and 4-week groups (n = 4/group), and (ii) the ultrasonic knife group, further divided into the 1- and 4-week groups (n = 4/group) (Fig. 1). General anesthesia was induced by a combination of butorphanol, midazolam, and medetomidine by intraperitoneal injection, followed by local anesthesia using lidocaine by subcutaneous injection in the surgical area. Hair clipping was performed outside the operation area using an electric clipper and hair removal cream. The skin was prepared using iodine, which was subsequently wiped off using 70% ethanol, which was sprayed on the surgical site. A 9-mm defect was created using a trephine bur (Implatex Co, Ltd.,

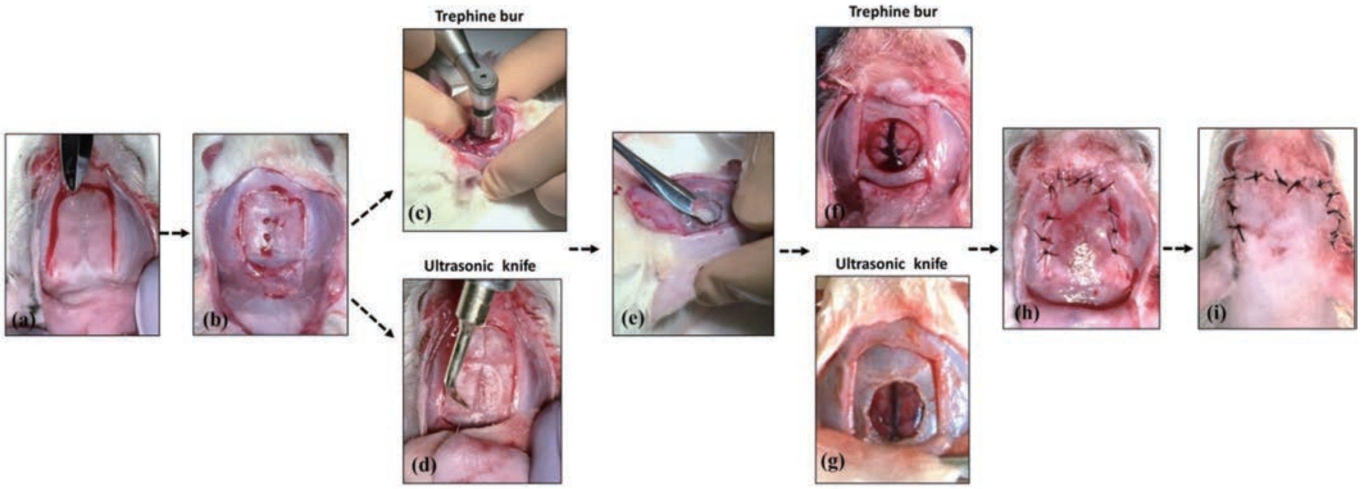

Figure 1. Surgical preparation of the defects. (a, b) U-shaped incisions on skin and periostin made to explore the calvarial bones; (c) bony defect is marked on the centre of the calvaria using a 9-mm trephine bur; (d) defect prepared using an ultrasonic knife with a scale-type cutting edge; (e) mucosal elevator was used to remove the bone fragments; (f) bony defect prepared using the trephine bur; (g) defect prepared using an ultrasonic knife; (h) periosteum was repositioned and sutured; (i) skin is closed.

Tokyo, Japan) or an ultrasonic knife (Sonic Surgeon 310 L, Dong IL Technology, Gyeonggi-do, Korea) with water injected in the center of the calvaria as a coolant. A mucosal elevator was used to remove the bone fragments. The periosteum was subsequently repositioned using a 5-0 suture, and the skin was repositioned using a 4-0 suture. The animals received a postoperative antibiotic regimen of gentamicin (GENTACIN®, MSD K.K., Tokyo, Japan) for two days (2 mg/kg/day). Further, four rats in the trephine bur group or ultrasonic knife group were injected with calcein after surgery (2 mg/kg; Wako Pure Chemical Industries Co., Osaka, Japan), which was continued for three days; these rats were euthanized at seven days after surgery. The other rats were euthanized using an overdose of pentobarbital sodium (2 ml/kg, Somnopentyl®, Kyoritsu, Tokyo, Japan) one month after surgery. The wounds were observed without signs of infection, dehiscence, or self-inflicted trauma.

Scanning electron microscopic observation

The cut edges of the surgical site that were made using a trephine bur or ultrasonic knife were observed using a field emission-scanning electron microscope (FE-SEM; 5-kV, S-4800, Hitachi High Technologies, Tokyo, Japan).

Bone histomorphometry

The samples were fixed in 4% paraformaldehyde for 24 h. The Kawamoto method was used to obtain four-micrometer-thick non-decalcified frozen sections[15]. The dynamic osteogenesis was studied by observing the fluorescently labeled sections from the 4-week group under an LSM700 laser-scanning microscope (Zeiss, Jena, Germany). To activate the fluorophores, lasers of different wavelengths were used, namely 488 nm (calcein, yellow) or 555 nm (Alizarin Red, red).

Histochemical staining and histological observations

Alkaline phosphatase (ALP) and tartrate-resistant acid phosphatase (TRAP) were stained for histological analysis. The TRAP and ALP staining were performed using the TRAP/ALP Kit (Wako Pure Chemical Industries Co., Osaka, Japan) to identify the osteoclasts and measure osteoblast activity. In our previous studies[16,17], we confirmed TRAP staining in frozen sections. So, we used the same method. After staining, the sections were observed using a BZ-9000 digital microscope (Keyence Co., Osaka, Japan).

Statistical Analysis

Statcel3 software (OMS, Tokyo, Japan) were used for the statistical analysis. For all experiments, values are reported as the mean ± standard deviation (SD). For comparisons between the two groups, the data were evaluated using Student's t-test. Statistical significance was set at $p < 0.05$.

Results

Operation time and bleeding volume

Although surgery using an ultrasonic knife requires more time to complete[18-20], no significant difference was observed between the trephine bur and ultrasonic knife groups in terms of bleeding volume and operation time because of the significant deviation in the trephine bur

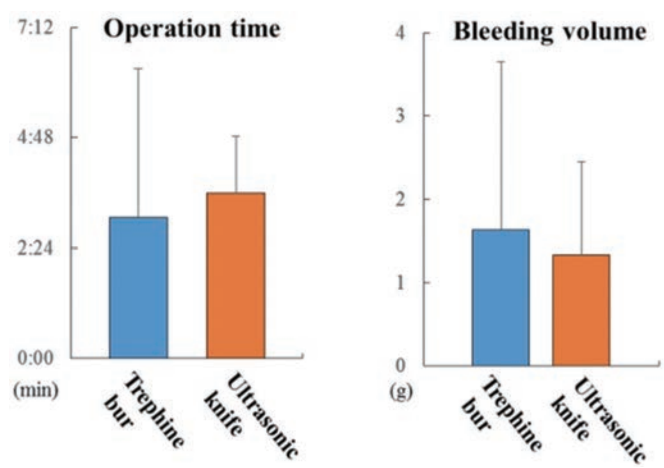

Figure 2. Quantification of operation time and bleeding volume of using trephine bur or ultrasonic knife. No significant difference could be found (SD; n = 8). Statistically significant difference at $p < 0.05$ (Student's t-test).

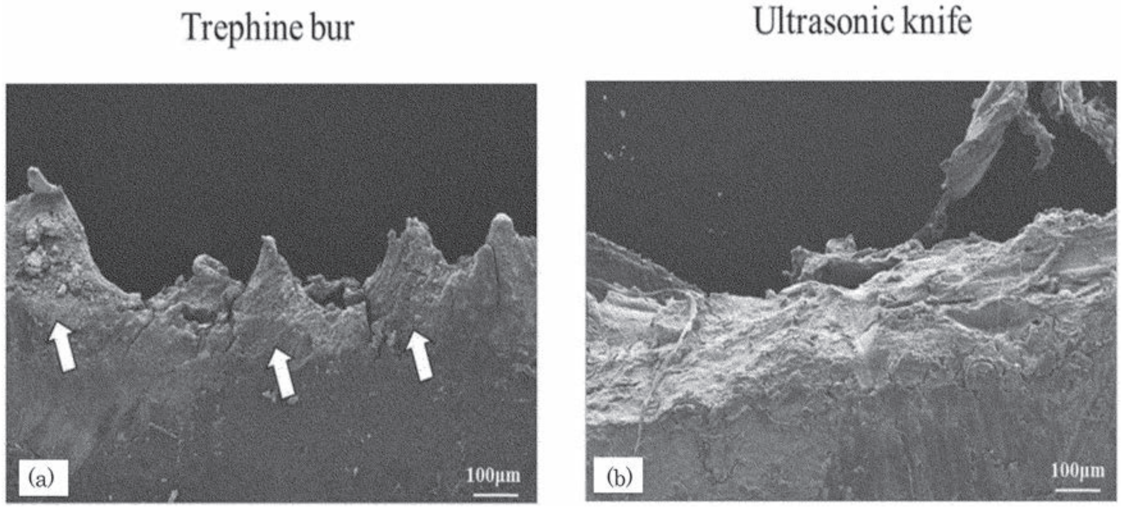

Figure 3. Scanning electronic micrographic images at the margin of the defects after preparation with trephine bur or ultrasonic knife (a, b).

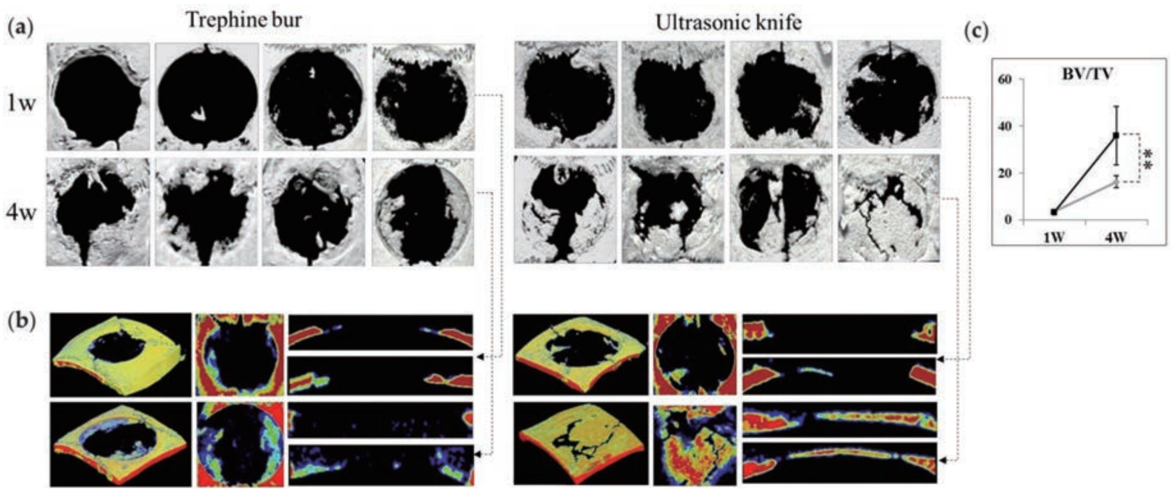

Figure 4. Critically sized bone defects in rat calvaria treated with the ultrasonic knife or trephine bur. Micro-computed tomography (a) and bone-mineral density (BMD) (b) images of the defects in the rat calvaria. (c) Postoperative bone volumes/tissue volumes (BV/TV). Black squares: Ultrasonic knife; Gray circle: Trephine bur. The data shown represent the mean ± standard deviation (SD; n = 4); ** $p < 0.01$ (Student's t-test).

group (Fig. 2). The standard deviation of the ultrasonic knife group was smaller than that of the trephine bur group.

SEM

In the SEM images of the trephine burr group (Fig. 3), the cutting surface of the bone defect was rough, with a large amount of bone debris (white arrows). However, in the ultrasonic knife group, the cutting surface was smooth.

Micro-computed tomography

We examined the bone morphometric changes that occurred after surgery (Fig. 4). In the trephine bur group, micro-computed tomography images and structural parameters of rat calvaria showed that the volume of the new bone formed on the bone defects in the ultrasonic knife group was greater than that formed in the trephine bur group. The trephine bur group showed a significantly lower average bone volume than the ultrasonic surgery group at four weeks ($p < 0.01$).

Fluorescence imaging

Fig. 5 shows the early bone formation identified using calcein (green) at the cutting surface of defects 1 week after surgery (Fig. 5). In addition, we found that different bone formations occurred on the cutting surface of the ultrasonic knife group, and the trephine bur group showed a reduced new bone formation compared to that in the ultrasonic knife group.

ALP/TRAP staining

We further evaluated the ALP/TRAP expression using histochemistry to assess the bone turnover capacity (ALP and TRAP staining for detecting osteoblast and osteoclast activation, respectively) in each group (Fig. 6 for ALP and Fig. 7 for TRAP staining). One week after treatment, the trephine bur group samples showed weak ALP expression in the cutting surface. In contrast, the samples from the ultrasonic knife groups were significantly stained black (ALP expression), indicating that the surgery using the ultrasonic knife could help retain bone-forming ability (Fig. 6). Further, the samples in the trephine bur group

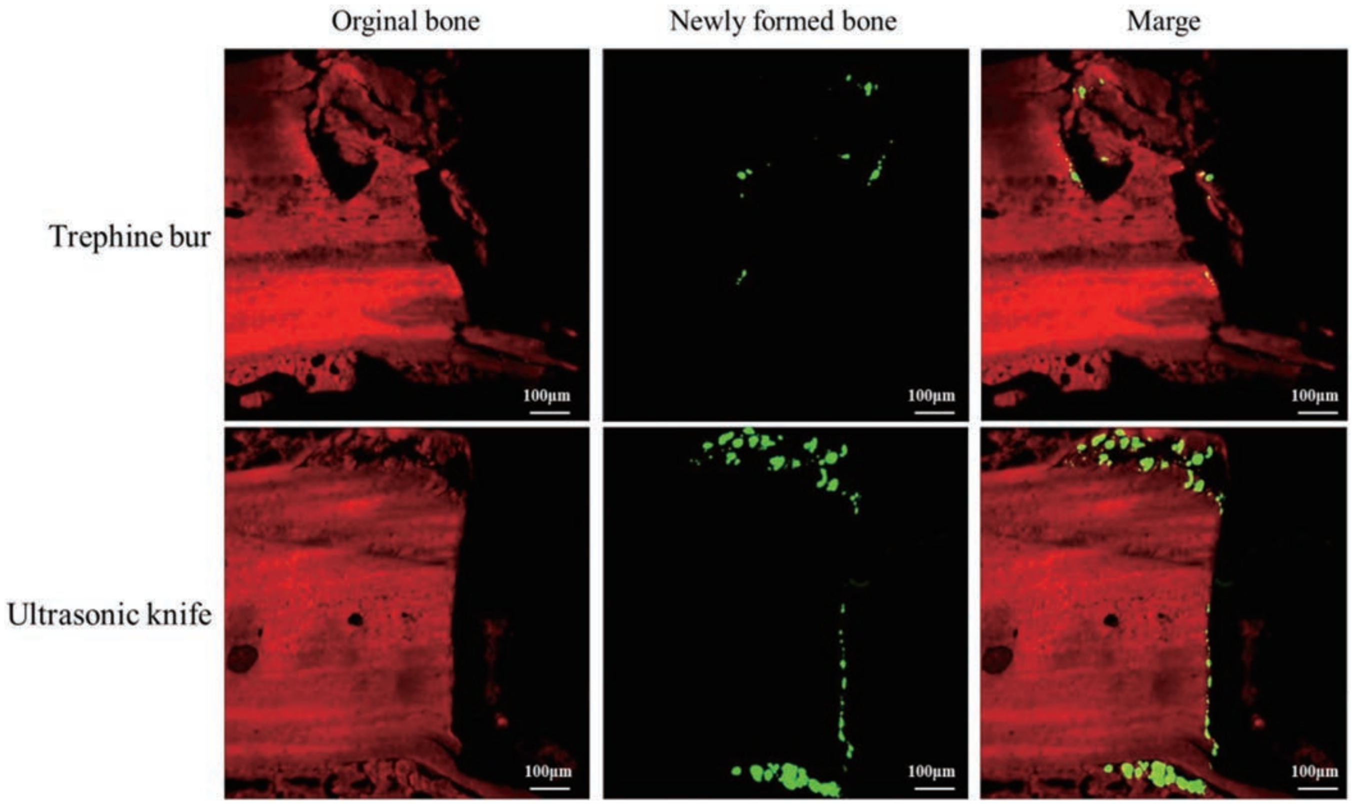

Figure 5. Fluorescence labeling analysis. Calcein (green staining: newly formed bone at 1-week post-implantation) and Alizarin red (red: contrastaining of calcified tissue) labeling of the bone tissue in the calvarial defects.

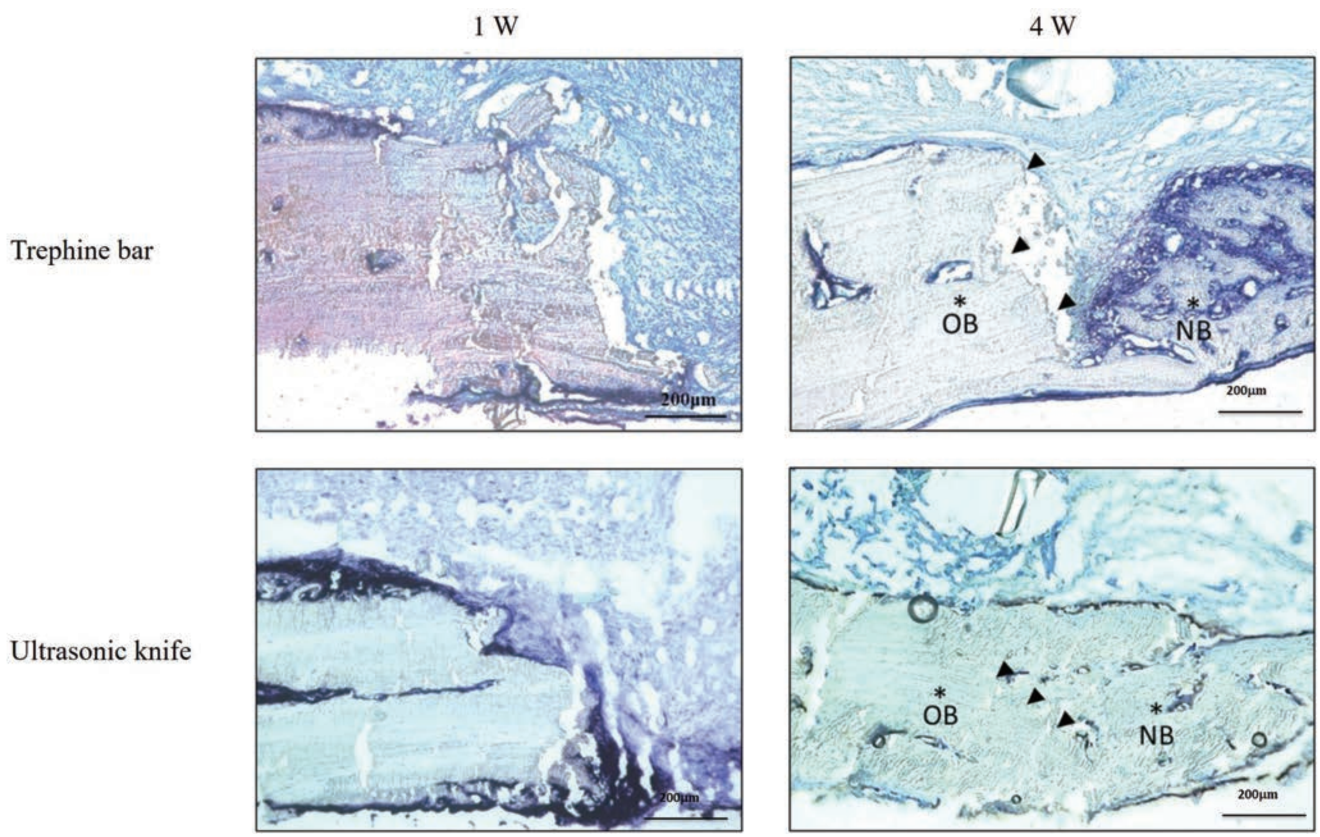

Figure 6. Alkaline phosphatase (ALP) staining; black stains represent ALP-positive tissue. OB: Original Bone, NB: New Bone.

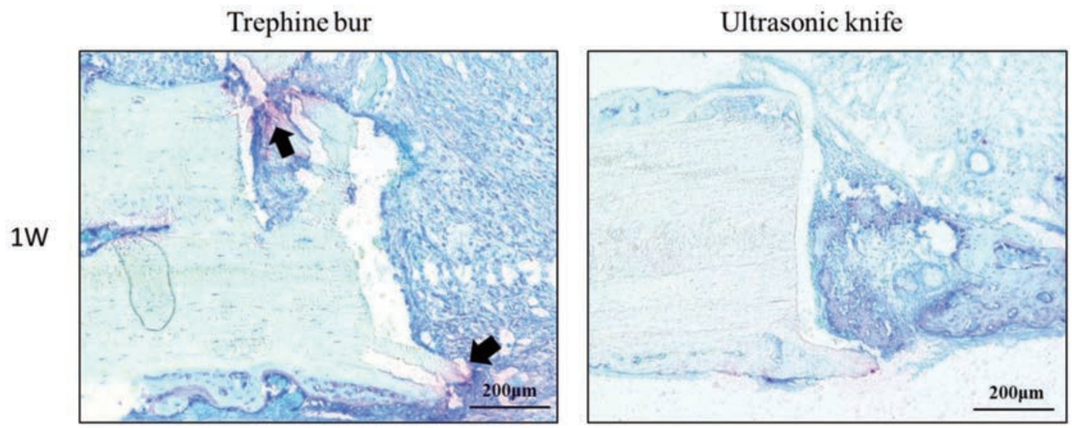

Figure 7. Tartrate-resistant acid phosphatase (TRAP) staining showing the presence of osteoclasts in the tissue sections.

showed significant TRAP staining (black arrow in Fig. 7) in the bone debris, suggesting that in cases of trephine bur surgery, bone resorption was promoted to remove the damaged bone tissue before the initiation of defect healing. This suggests surgery using an ultrasonic knife may help attenuate damage to the mother bone, leading to bone regeneration being initiated earlier than in cases where the trephine bur is used.

Discussion

Some studies comparing the efficacy of osteotomy using an ultrasonic knife and with that of conventional techniques using a trephine bur for bone healing have reported no differences in the postoperative bone formation[21,22]. However, in the present study, we demonstrated that critical-size bone defects formed with a trephine bur and an ultrasonic knife induced bone formation at different speeds. Postoperative bone formation in cases of ultrasonic surgery was significantly greater and began earlier than that in cases of surgery performed using a trephine bur in a rat model.

In our experimental rat model, the differences in bleeding or operation time between osteotomies prepared by ultrasonic knife and conventional rotary burs failed to reach statistical significance (Fig. 2). However, this may be because the surgeon using the trephine bur must have been cautious in avoiding damage to the dorsal cerebral vein. Generally, if the trephine bur comes in contact with the dorsal cerebral vein, it causes severe hemorrhage, which is difficult to control during the surgery, which increases the duration of the surgery. In addition, damage to the dorsal cerebral vein, which causes large blood clots and swelling, is likely to influence bone repair progress. However, the narrow and long ultrasonic knife provides a clearer and unobscured view of the surgical field, including delicate vein structures, which can be used to circumvent severe hemorrhage.

In clinical practice, osseointegration to the implant is affected by the surface structure of the implant body and the condition of the surface of the implant bed[12]. It is believed that protecting the surface of implant bed from damage equally important[23]. In the present study, at the cutting surface of bone defects treated with trephine bur, we observed more bone fragments, suggesting that increased damage was caused to the bone surface compared to that in ultrasonic knife surgery. In cases where the trephine bur was used, osteoclastic absorption of the bone fragments to create the optimum environment was osteoblastic bone regeneration is likely to take some time. In contrast, a smaller amount of bone debris was observed at the cutting surface in cases of ultrasonic knife than that in trephine bur surgery, suggesting that the former technique causes reduced damage to the bone defect surface (Fig. 3). These results indicate that the early initiation of bone healing in cases of ultrasonic knife surgery can be attributed to reduced damage to the mother bone.

Vajgel *et.al.* reported a systematic review that showed that when using the conventional rotary drill, the mean expected new bone formation in 9.0-mm-diameter calvaria defects in the rat model was 11.18% of that at one month after surgery[24]. The mean bone formation can range from 4.93%–30%, depending on the surgeon. In the present study, the defect prepared using ultrasonic surgery was 35.1% one month after surgery. Additionally, we believe that the ultrasonic knife reduced the risk of damage to the soft tissues when compared with that of the round bur[25]. Considering these benefits, the ultrasonic knife may be a better candidate for osteotomy, to help induce earlier bone formation. However, in our study, one surgeon performed all the osteotomies. The deviation of bone regeneration was 19.7%–53.1% when an ultrasonic knife was used without any bone grafts or bone substitutes. It is believed that the skill of a surgeon significantly influences the quality of surgery. Further detailed investigations involving more surgeons are required to conclusively prove the superiority or inferiority of these instruments.

The experimental model with a critical-sized bone defect in calvaria is a well-used model in mice, rats, rabbits, and canines to detect the effect of certain devices[26-29] and elucidate the bone-forming ability of autogenous bone grafts or of various biomaterials[30-33]. In this study, bone defects formed using the trephine bur showed a reduced deviation than those formed using an ultrasonic knife (Fig. 4), suggesting that a trephine bur is likely to be a suited for the preparation of a severe and consistent bone defect model in rat calvaria when comparing various materials. Our results indicate that careful attention should be paid to the surgical devices before comparing the results of bone formation in different studies, even for bone defects of the same size; the use of different surgical devices may alter the basement level of bone formation.

In conclusion, this study shows that bone regeneration in rat calvaria can be enhanced when using an ultrasonic knife in preparing osteotomy defects compared to using trephine bur under similar surgical times. The increased bone formation in cases of ultrasonic knife surgery may be due to the reduced damage to the mother bone, possibly due to the sustained bone-forming activity of osteoblasts. In addition to other advantages of ultrasonic knives, such as minor damage to soft tissues, the results of our study suggest that ultrasonic knife surgery may be preferable to conventional osteotomy surgery in clinical settings.

Conflicts of Interest

The authors have declared that no COI exist.

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