



An experimental comparative study of drilling efficiency and temperature elevation with unmodified and modified medical drills in pig tibia bone



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ABSTRACT

Background: There are no medical drill specifications capable of achieving bone drilling in a short time under low-thrust and low-speed drilling. Gekkou-drill® is an industrial drill that enables drilling with low cutting resistance by its characteristic point design. Our aims were to develop Gekkou-modified drills by processing to the points of currently available medical drills and to verify whether these modified drills enable less invasive drilling procedure for bone tissue in thermal exposure compared with unmodified medical drills.

Materials and methods: Two commercially available 3.2-mm drills were compared before and after Gekkou modification. Drilling of pig tibias was performed at speeds of 300, 800, and 1,500 rpm and a uniform thrust force of 10 N. Temperature at the entry point for bone drilling was measured using a digital thermometer system. The feed rates were calculated using cortical thickness and monitoring data of the digital force gauge.

Results: Two unmodified drills could not penetrate the cortical bone on the near side at 300 rpm, even after 5 min of drilling. The maximum temperatures with modified drills were 54.6 °C and 46.2 °C at 300 rpm. At medium to high speeds, those were statistically significantly lower than with unmodified drills (58.5 °C vs. 90.5 °C at 800 rpm, 62.6 °C vs. 80.8 °C and 73.9 °C vs. 104.6 °C at 1,500 rpm). The feed rates for modified drills were 4.9–6.9 times as high as unmodified drills at 800 rpm, and 3.4 to 4.5 times at 1,500 rpm. On the other hand, the feed rates of modified drills at 300 rpm were equal to or higher than those of unmodified drills at 1500 rpm.

Conclusion: Gekkou-modified drills clearly suppressed the temperature rise and increased the feed rate compared with conventional drills. Furthermore, it was notable that these modified drills had higher performance even at conditions of low thrust and low speed.

1. Introduction

Bone drilling procedures can cause both mechanical damage and thermal trauma to bone tissue. Thermal damage to living tissue is related to the magnitude of the temperature increase and the duration of tissue being exposed to damaging temperatures [1, 2]. The inverse relationship between the temperature at which necrosis occurs and time to necrosis (44–100 °C) was studied for a wide temperature range; an increase in temperature above 70 °C causes immediate damage of epithelial cells in the drilling tract [3, 4]. On the other hand, the exact temperature threshold for human bone death due to overheating is currently unknown [5, 6]. However, most researchers believe that an average temperature of 47 °C for 1 min is the threshold above which necrosis of human bone will occur [1, 6, 7, 8, 9, 10].

Parameters influencing the temperature rise during bone drilling can

be broadly divided into two groups: drilling parameters and drill specifications. Regarding drilling parameters, many researchers have studied the influence of drilling speed (revolutions), thrust force (axial drilling force), and feed rate on bone drilling temperature. All the parameters listed here are directly involved in drilling energy, which affects thermal damage to bone. During bone drilling procedures, thermal damage to bone increases with an increase in the number of drilling speed, thrust force, or feed rate [11]. Ideally, drilling should minimize mechanical and thermal damage to bone tissue under conditions of short duration, low thrust force, and low drilling speed. Unfortunately, to date, there are no medical drill specifications capable of achieving bone drilling in those conditions. Currently, the most effective means to reduce thermal damage to bone is drilling under conditions that enable the completion of drilling in a short time, that is, a high speed and high thrust force suppress heat generation [12, 13, 14, 15, 16, 17].

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Since the development of drills with special equipment such as coolant systems, the development of minimally invasive drills has been stagnant. On the other hand, in industry, an innovative drill has been frequently used to improve working efficiency during drilling by drastically reducing cutting resistance and reducing cutting heat. This drill is sold under the brand Gekkou-drill® [18]. It has recently been drawing attention as a new-generation drill that improves working efficiency and safety. The special features of the Gekkou-drill® are based on the unique shape at its drill point, which is considered to be the main cause of reduced cutting resistance during drilling. We had the opportunity to develop the medical drills modified to a special drill point shape of Gekkou-drill® and evaluate the performance of them in *in vitro* experiments. Our aim was to compare Gekkou-modified drills with the unmodified commercially available medical drills which are commonly used in terms of drilling efficiency and thermal effects.

2. Materials and Methods

We evaluated two different 3.2-mm bone drills, which were the most current versions at the time of testing, for the Gekkou modification (Fig. 1). Drill A (Johnson & Johnson Inc., New Brunswick, NJ, USA) and drill B (Zimmer Biomet Inc., Warsaw, IN, USA) were both commercially available twist drills with two helical flutes. We applied Gekkou modifications to the drill points of drills A and B to yield drills AG and BG, respectively. The Gekkou process point design had a crescent-shaped cut (end view) for each cutting lip and was grounded with a point angle of 55° for AG and 60° for BG. The clearance angle was 28° for AG and 15° for BG. All the drills tested were modified by the same manufacturer (BIC TOOL Co., LTD, Hiezu, Tottori, Japan).

Cortical bone specimens demonstrate large interspecies variations. Pig bones best resemble human samples [19]. In order to retain their mechanical and thermophysical properties, the specimens were kept moist in saline solution and stored in plastic bags at -10 °C [20]. However, the slaughterhouse (Shibaura Slaughterhouse, Tokyo Metropolitan Government) was far from our laboratory. Therefore, when not used immediately, specimens were prepared according to the method of Sedlin and Hirsch and used within 2 days after slaughter. All specimens came from females or castrated males that were 6 months old and 100–120 kg in weight. The central part of tibial diaphysis of the hind legs (60 mm) was used. The cortical thickness was 2.7 ± 0.1 mm (from 2.5 to 3 mm). All tests were performed at room temperature (24–26 °C). Initially, the temperature of the bone was set at 31 ± 1 °C.

The drilling parameters in common surgical procedures, high speed and high thrust force, are set so that the bone drilling can be completed in a short time irrespective of the drill specification. Therefore, with conventional surgical drilling parameters, there is a high possibility that the performance difference depending purely on the drill specification is hidden, and it is difficult to evaluate them properly. In this verification, the condition that purposely makes it difficult to finish drilling in a short time, that is, low thrust force is set as one of drilling parameters. The experimental setup is shown in Fig. 2. Bone drilling was performed on a dynamic material testing system, which can provide adjustable drilling speeds with a uniform thrust force (10 N) using the digital force gauge (RZ-20, Aikoh Engineering Co., Ltd, Osaka, Japan). Thrust force during each bone drilling procedure was monitored over time and data was preserved (Fig. 3). At the same time as drilling starts, the thrust force value becomes constant at 10 N and decreases at the moment when it penetrates the near side and contralateral side of cortical bone. It was possible to calculate the period to penetrate the cortex from each point. During each experiment, the drilling speed was fixed at 300 revolutions per minute (rpm), 800 rpm, or 1,500 rpm. Although the drilling speed set in the orthopedic surgical procedure is mostly 1,000 rpm or more, sometimes the initial drilling is performed at several hundred rpm in the procedure of attaching a footprint mark to the slippery bone surface. The number of revolutions used in that initial drilling procedure was set as low-speed drilling that is actually used in orthopedic surgery, and in this

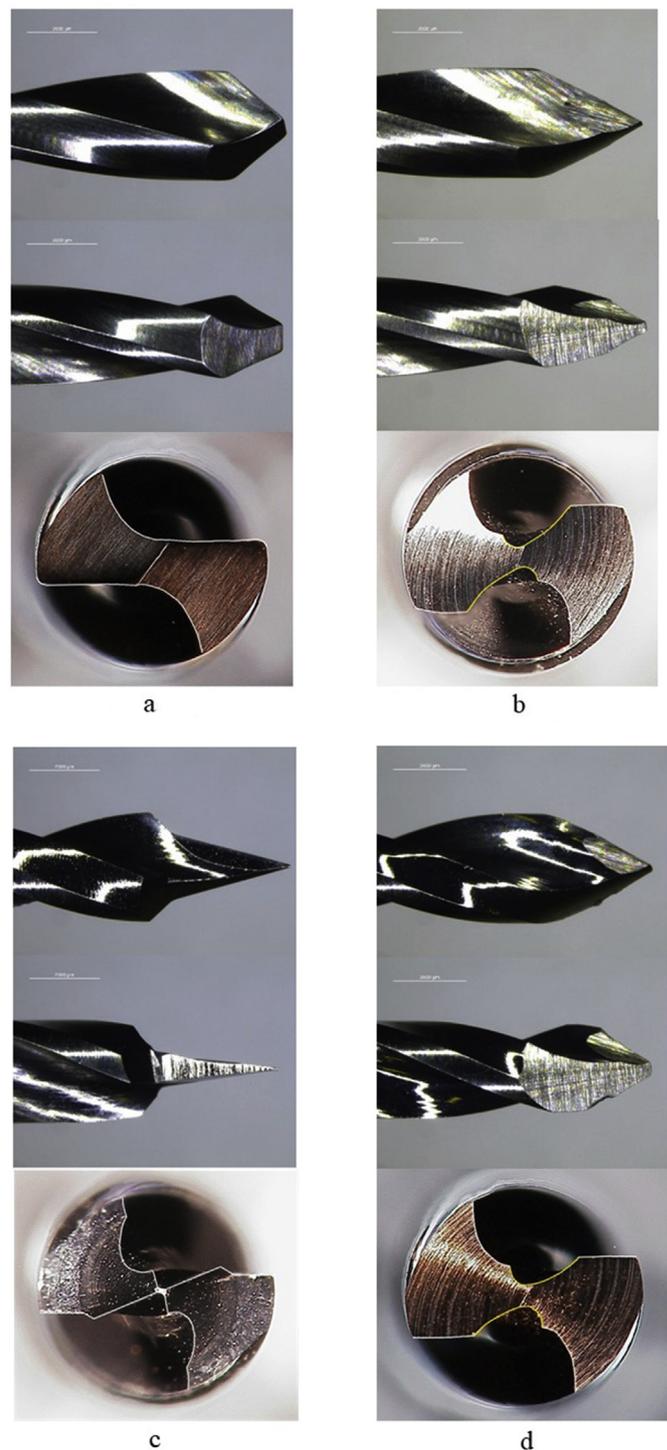


Fig. 1. (a) Drill A (Johnson & Johnson). (b) Drill AG (Gekkou-modified Drill A). (c) Drill B (Zimmer Biomet). (d) Drill BG (Gekkou-modified Drill B). The yellow line shows the crescent-shaped cutting line in end-view photographs of (b) and (d).

verification, the revolution number was set to 300 rpm. In addition, as the rotation speed frequently used for bone drilling in orthopedic surgery, the medium drilling speed number was set to 800 rpm and the medium to high speed number was set to 1500 rpm, which was applied in this experiment. In the preliminary experiment of bone drilling using the drill A and B in the pig tibia, the proper thrust force was verified. As a result, when drilling perpendicularly to the bone surface at each revolution number (300, 800, 1,500 rpm), the averages of 5 times of the

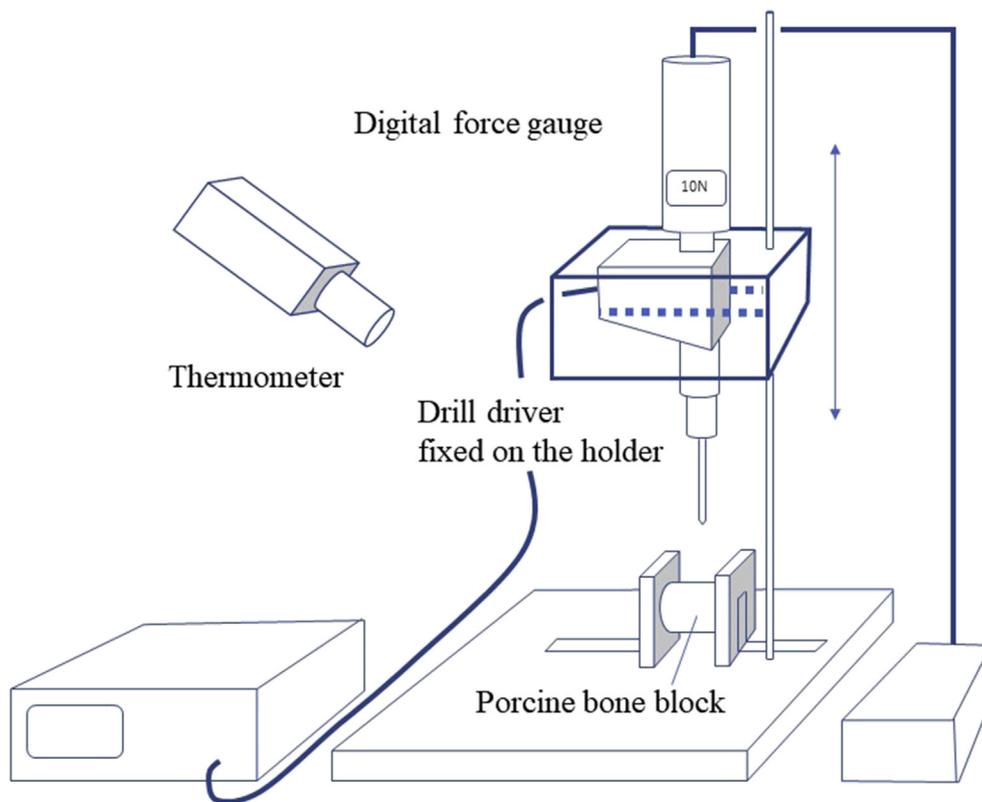


Fig. 2. Diagram of the experimental setup. Drilling was performed on a dynamic material testing machine, which can provide adjustable drilling speeds with a uniform thrust force (10 N) using a digital force gauge. Infrared thermography was used to measure temperature changes.

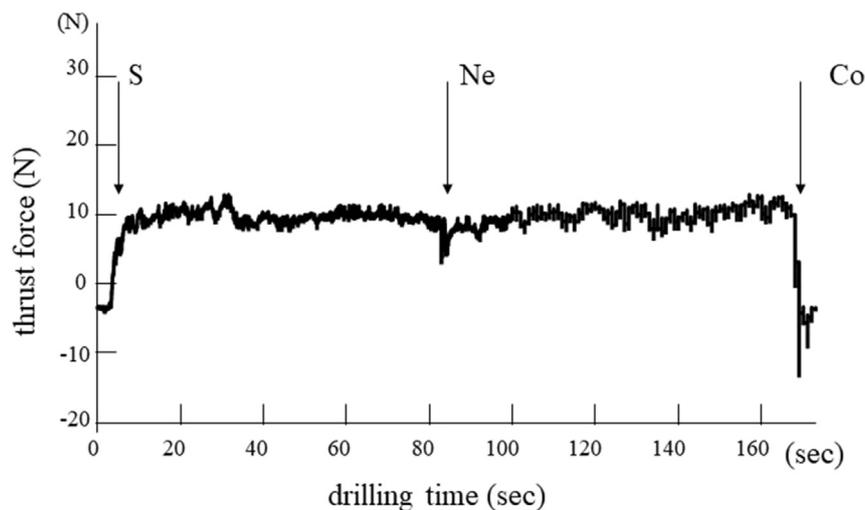


Fig. 3. Monitoring data of digital force gauge with drill B at 800 rpm. S, at start drilling; Ne, at the near side of cortical bone penetration; Co, at the contralateral side of cortical bone penetration.

minimum thrust force where the drill point did not deviate by 1 mm or more were 8 ± 1 N for drill A and 8.5 ± 1 N for drill B. Based on the above, the thrust force was fixed to 10 N as a drilling parameter that can initiate minimally invasive and stable bone drilling.

Bone samples were clamped in a vise fixed on the platform of the testing machine. A regular hand drill, connected to a flexible drive rod, was used to transfer the rotational torque to the drill bit holder mounted to the testing head of the machine. For each type of point, four drills were used and each was tested six times. Temperature elevation during drilling was measured using a visual infrared thermometer (FLIR T650sc, measurement accuracy ± 1 °C or $\pm 1\%$, FLIR Inc., Wilsonville, OR, USA). The

region of interest was set on the monitor of the thermometer at the upper surface of the cortical bone specimen where bone drilling began (Fig. 4a, c). In addition, the temperature of the drill point was recorded when it returned to the upper surface of the cortex (the interest region of the thermometer) immediately after penetration of both cortices. The respective cortical penetration time and speed (average feed rate) were calculated with the force gauge monitoring data and the measured cortical thickness. Data were analyzed using descriptive statistics. The Kruskal-Wallis test and post hoc Scheffé test (confidence level, 95%) were used. Data analysis was performed with SPSS statistical software (version 20; IBM Corp., Armonk, New York, USA).

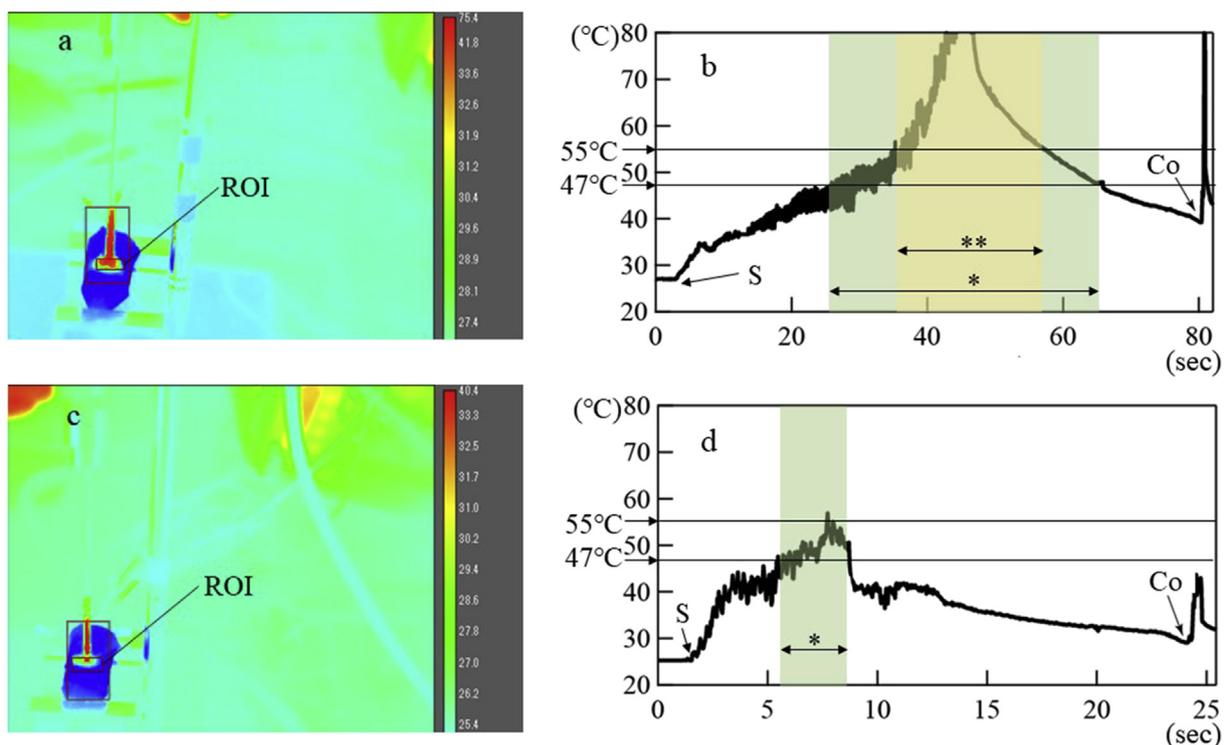


Fig. 4. Region of interest (ROI) of bone surface in thermometer image: (a) with drill B at 800 rpm and (c) with drill BG at 800 rpm. Maximum temperature of ROI and thermal exposure time generated during bone drilling: (b) monitoring graph with drill B at 800 rpm and (d) with drill BG at 800 rpm. The duration of thermal exposure over 47 °C (*) and over 55 °C (**) during bone drilling. S, at start drilling; Co, at the contralateral side of cortical bone penetration.

3. Results

Compared with the commercially available drills A and B, the modified drills AG and BG took significantly less drilling time to penetrate the near and contralateral sides of cortical bone under all conditions (Table 1, Video 1 and 2). In particular, at 300 rpm, which is a low drilling speed, drills A and B could not penetrate the cortical bone on the near side, even after 5 min of drilling. On the other hand, at 300 rpm, drills AG and BG were able to penetrate both cortices in half the time or less than it took drills A and B at 1,500 rpm. The maximum temperature of the bone surface with drill AG (62.6 °C) was statistically significantly lower than with drill A (80.8 °C) at 1,500 rpm. Additionally, maximum temperatures with drill BG (58.5 °C at 800 rpm, 73.9 °C at 1,500 rpm) were statistically significantly lower than with drill B (90.5 °C at 800 rpm, 104.6 °C at 1,500 rpm) in comparisons with the same drilling speed conditions (Table 2). The drill point temperature just after penetrating both cortices showed similar results.

The feed rate (mm/sec) of each experimental condition was calculated based on the measured thickness and cortical bone drilling time on the near side. Feed rate increased with a rise in the number of revolutions, except that there was no data with commercially available drills A and B at 300 rpm because they could not penetrate the near side of the

cortical bone at this speed. The feed rate of drill AG was remarkably affected by the number of drilling revolutions (Fig. 5). Both drills AG and BG had significantly higher feed rates than drills A and B at the same number of revolutions. The feed rate ratio for drill AG/drill A was 4.9 at 800 rpm and 4.5 at 1,500 rpm. For drill BG/drill B, it was 6.9 at 800 rpm and 3.4 at 1,500 rpm. The feed rate for drill AG at 800 rpm was significantly higher than that of drill A at 1,500 rpm. Similarly, the feed rate of drill BG at 300 rpm was also significantly higher than that of drill B at 800 rpm. The feed rate of drill BG at 800 rpm was significantly higher than that of drill B at 1,500 rpm.

Drilling might cause thermal damage to bone tissue, leading to osteonecrosis. Based on two representative studies [5, 6], we defined the threshold at which osteonecrosis occurs at 47 °C for 60 s and at 55 °C for 30 s. The experimental results were verified with the parameters of bone surface temperature and thermal exposure time generated during bone drilling (Fig. 4). Drilling with drill B at 800 rpm and 1,500 rpm exposed bone tissue to temperatures above 47 °C for over 60 s (Fig. 6). The duration of thermal exposure over 47 °C during bone drilling with drills AG and BG was significantly shorter compared with unmodified drills under the same conditions. Similar results were obtained with exposure to temperatures above 55 °C for over 30 s (Fig. 7). In particular, with drill AG, thermal exposure time was remarkably shorter at all drilling speeds.

Table 1
Time needed to penetrate bone with different combinations of drilling parameters.

	Drill speed (rpm)	Drill A Mean ± SD	Drill AG Mean ± SD	P value	Drill B Mean ± SD	Drill BG Mean ± SD	p value
Time to penetrate the first cortex during drilling (seconds)	300	–	14.3 ± 2.6	–	–	15.4 ± 2.1	–
	800	31.2 ± 12.2	5.8 ± 1.5	<0.0001	79.0 ± 32.2	9.8 ± 1.3	<0.0001
	1500	15.6 ± 7.5	3.2 ± 0.4	0.005	47.7 ± 36.5	10.0 ± 4.7	0.01
Time to penetrate both cortices during drilling (seconds)	300	–	31.3 ± 8.3	–	–	33.8 ± 8.8	–
	800	56.2 ± 20.4	12.8 ± 1.9	<0.0001	150.4 ± 57.6	28.0 ± 7.2	<0.0001
	1500	27.6 ± 13.8	6.2 ± 0.8	0.007	79.8 ± 46.7	20.6 ± 7.7	0.01

rpm, revolutions per minute; SD, standard deviation.

Table 2
Maximum temperature (°C) with different combinations of drilling parameters.

	Drill speed (rpm)	Drill A Mean ± SD	Drill AG Mean ± SD	p value	Drill B Mean ± SD	Drill BG Mean ± SD	p value
Maximum temperature of bone surface (°C)	300	–	54.6 ± 7.1	–	–	46.2 ± 1.4	–
	800	69.6 ± 8.1	60.7 ± 3.1	0.13	90.5 ± 2.7	58.5 ± 1.9	<0.0001
	1500	80.8 ± 11.5	62.6 ± 3.8	0.002	104.6 ± 21.7	73.9 ± 3.2	<0.0001
Maximum temperature of drill point (°C)	300	–	53.3 ± 4.0	–	–	44.8 ± 4.7	–
	800	57.1 ± 3.2	58.4 ± 3.0	0.80	63.0 ± 10.8	51.5 ± 8.2	0.01
	1500	65.3 ± 12.2	53.1 ± 7.4	0.017	81.5 ± 5.8	56.7 ± 3.4	<0.0001

rpm, revolutions per minute; SD, standard deviation.

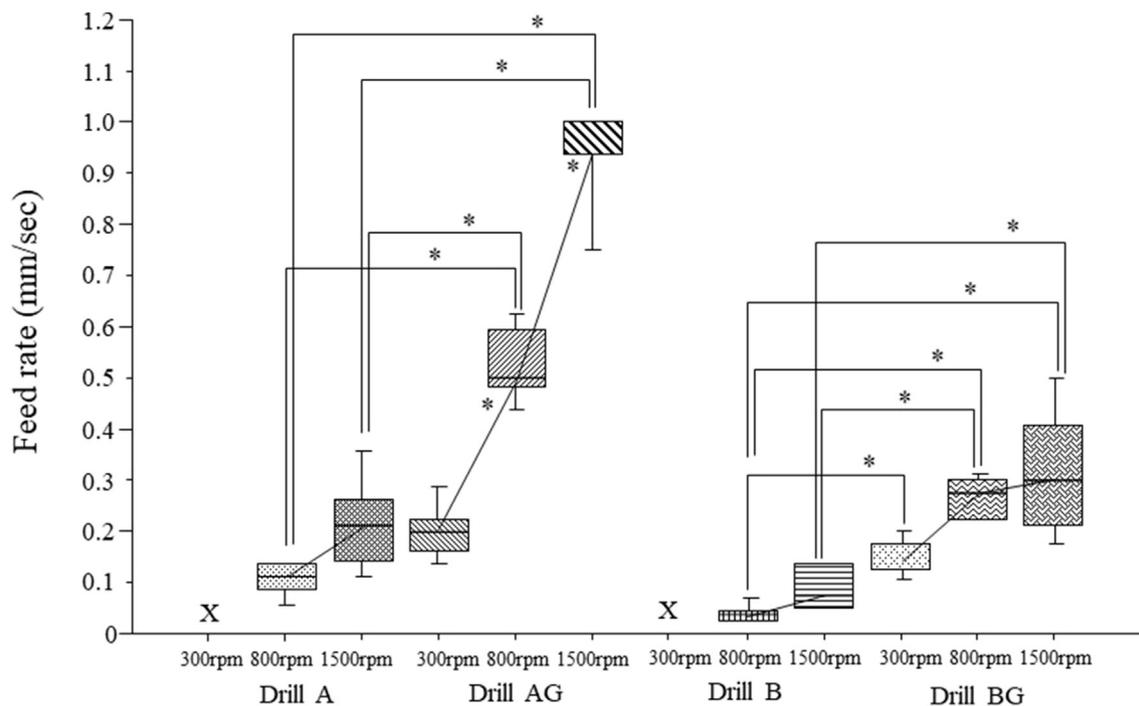


Fig. 5. Feed rate for each drill specification with each drilling speed during penetration of the first cortex. X shows that drill A and B were not able to penetrate the first cortex. sec, second; rpm, revolutions per minute.

4. Discussion

Ideally, drilling should minimize mechanical and thermal damage to bone tissue under conditions of short duration, low thrust force, and low drilling speed. To the best of our knowledge, there have been no previous studies that have verified a temperature reduction and high feed rate during drilling with a modified drill under conditions of low thrust force and low drilling speed. In this *in vitro* experiment, we verified the efficiency of two modified 3.2-mm diameter drills in bone drilling, showing not only that the temperature rise can be reduced but also that drilling can be completed in a short time under low thrust force and low speed conditions. This report is likely the first demonstration of ideal drilling conditions only on the basis of drill specifications, without using special equipment such as a cooling system. In this experiment, factors that enabled drilling under conditions considered to be gentle to bone tissue include the characteristic point design combined with a somewhat steep point angle and crescent moon cutting applied to the cutting edge with the Gekko modification. Compared with commercially available standard bone drills, there was no change in the drill material and specifications other than the point angle and cutting edge.

Drill specifications are major factors influencing heat generation during drilling [12, 13, 14, 15, 16, 17]. A drill is usually characterized by its diameter, cutting face, helix angle, and drill point. The drill cutting face is further specified by the rake angle and clearance angle, whereas

point angle, flank, and chisel edge define the drill point. Many researchers recommend that the point angle of the drill should be approximately 90°–130° [17, 21, 22, 23]; within this range, there is no significant difference in temperature rise during bone drilling [24]. There is no general agreement on the optimal drill point angle. There have been no reports describing the direct involvement of the chisel edge in thermal damage during bone drilling. However, many reports have demonstrated that a reduction in the chisel edge leads to a lower thrust force and shorter drilling duration, which leads to a lower temperature rise during bone drilling [17, 23, 24]. Sannino compared friction heat during bone cutting of two drill shape designs that differed only in the length of the cutting surface of the drill. That study indicated that reduction in length of the cutting surface of the drill may limit frictional heat [25]. Koo had used three types of drills in study such as titanium nitride-coated metal, tungsten carbide carbon-coated metal, and zirconia ceramic drill to evaluate the effects of drill wear on bone temperature during osteotomy preparation and there was no significant difference between the drill materials [26].

The Gekko drill modification used in this study affects the point design, as mentioned above. Specifically, the point angle is 55° for AG and 60° for BG, the helix angle is 16° for AG and 24° for BG, and the clearance angle is 15° for both AG and BG. The cutting edge of both drills was applied to crescent moon cutting. The point angle is somewhat steeper than with commercially available bone drills, but this

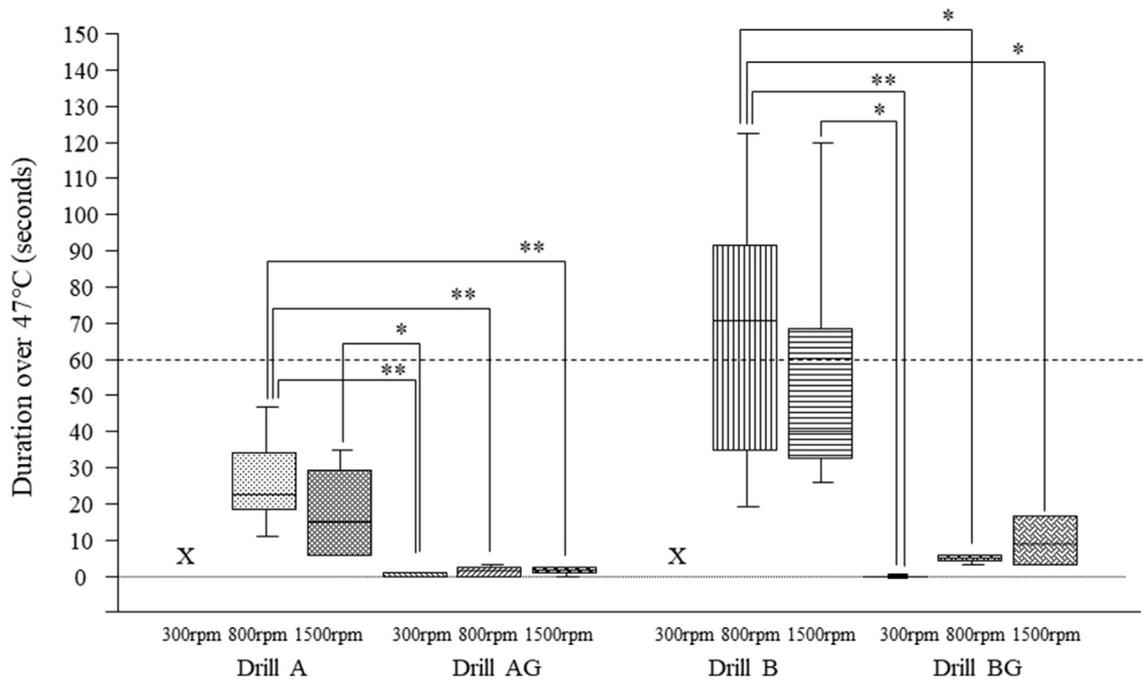


Fig. 6. Effect of drill specifications and drilling speed on the duration of temperature elevation above 47 °C recorded at the drilling bone surface. X shows that drill A and B were not able to penetrate the first cortex. rpm, revolutions per minute.

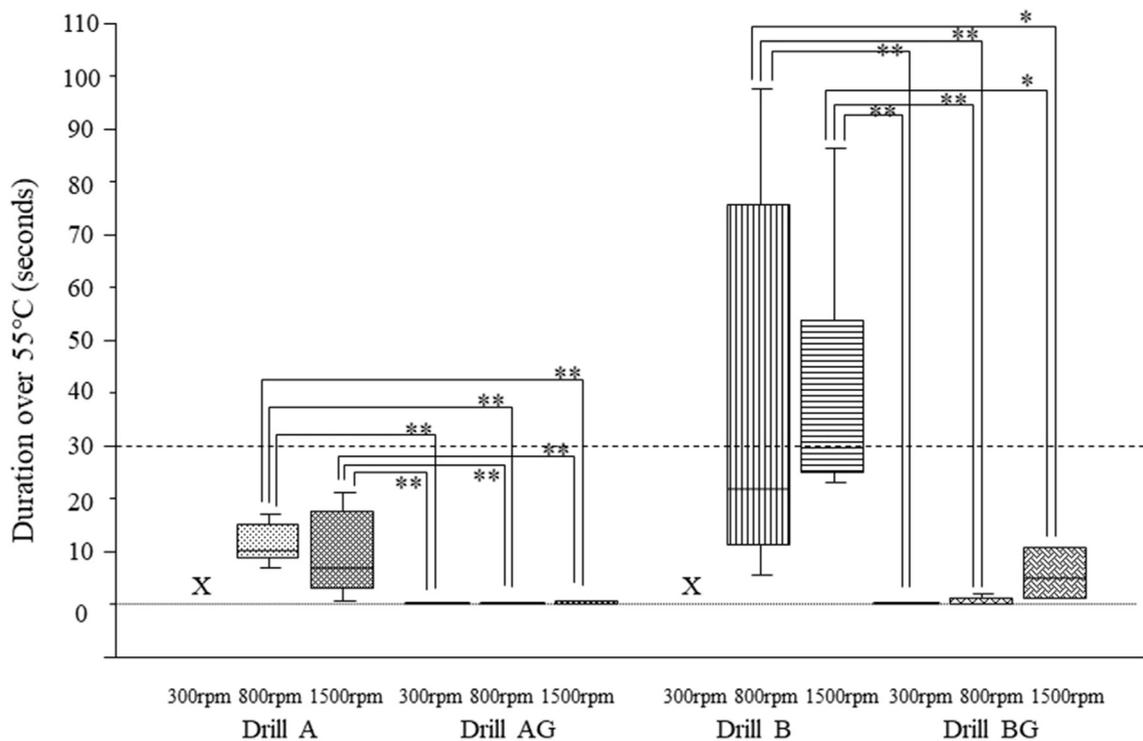


Fig. 7. Effect of drill specifications and drilling speed on the duration of temperature elevation above 55 °C recorded at the drilling bone surface. X shows that drill A and B were not able to penetrate the first cortex. rpm, revolutions per minute.

specification did not have a negative influence on cutting ability. In this medical drill experiment, the purpose of reducing the point angle with the Gekkou modification was to add another excellent feature to this drill: by decreasing the point angle, the oblique insertion angle to the bone surface is expanded, and even under conditions of a strong oblique position, it does not reduce the performance as it prevents slipping, another characteristic of this modification. Modifications of the drill used

in this study, including crescent moon cutting, which can be said to be the most essential feature, were carried out by BICTOOL Co., LTD, which has no funding relationship with this study. Drills with crescent moon cutting modifications have already been marketed for industrial use [18]. They are attracting attention as excellent drills with minimal cutting resistance and are frequently used. This experiment demonstrated that a reduction in cutting resistance with Gekkou modifications can reduce thermal

damage to bone tissue for medical and industrial drills. Although details on the mechanism of cutting resistance reduction have not yet been proven technically, it is clear that crescent moon cutting of the cutting edge was responsible. In addition to reducing the chisel edges, it is presumed that the sharp edges have a rounded scoop shape, which worked favorably for a smooth drilling operation. In other words, the former enabled drilling with a low thrust force, and the latter is thought to reduce cutting resistance during drilling.

Less number of literatures are there showing importance to the amount of pressure and the resulting frictional heat generated. According to Brisman, the force applied on the hand piece was more influential than the speed of the drill in temperature elevation. They found that the drill speed was not the critical determinant of heat production, rather the difference in the drilling force was related to both the maximum temperature elevation and periods of temperature elevation. Increasing both the speed and the load together allowed for more efficient cutting with no significant temperature increase [27]. Abouzgia also suggested that drilling at a high speed and with a larger load was more efficient than using low speed and a lesser load [28]. However, although the currently recommended drilling operation makes sense in terms of reducing the thermal exposure time, it is not an ideal minimally invasive operation in terms of conditions that are inherently highly invasive (high speed and high thrust force). Therefore, we thought that if drill specifications with minimal cutting resistance and large feed rate are available, bone drilling can be performed in a short time even under minimally invasive conditions (low speed and low thrust force). As a preliminary experiment, we verified bone drilling under high speed and large thrust force using Gekkou-modified drills, and as a result, bone drilling was possible in a significantly shorter time than commercially available standard bone drills. In this research, verification was conducted under low speed and low thrust force conditions in order to reduce the invasiveness. Bone drilling with low thrust force by the Gekkou-modified drills significantly reduced the temperature rise and thermal exposure time as compared to procedure of conventional drilling conditions (high speed and high thrust) by commercially available drills. The present results suggest that not only the drilling conditions but also the drill specifications themselves have the potential to realize less invasiveness.

Saline irrigation is mostly used for the prevention of the heat generation during osteotomy for the protection of the bone from the thermal damage [29, 30, 31, 32]. And also, most of the surgeons prefer cool saline solutions with the belief that they are more effective than the normal solutions for the reduction of the temperature. On the other hand, there have been reports of unconventional drilling techniques aimed at reducing thermal exposure during bone drilling. Gabrić had done study to compare thermal changes after drilling with an Er: YAG laser versus a low-speed surgical drill [33]. The temperature was statistically lower during the laser preparation. Cavities prepared with the laser were regular with clear sharp edges and knife-like cuts, with regular and sharp edges, without bone fragments and debris which resulted in lesser generation of heat in a shorter period of time. Zheng reported ultrasonic osteotomy and drilling as a special instrument [34]. The main advantages of ultrasonic techniques include the selective cutting of hard tissue, the hemostatic effect on the surrounding tissue, and the generation of a gentle, precise cut without the need for excessive force. In this study, although examination using the above-mentioned irrigations and unconventional techniques has not been conducted, further studies are needed to enable further minimally invasive bone drilling by adding the advantage of these drilling techniques and tools.

The present study has several limitations. First, temperature measurement was limited to the bone surface and the drill point during drilling. Most studies of thermal damage during bone drilling use direct temperature measurement with a thermocouple sensor [35, 36, 37] or thermography to measure the temperature of a region of interest [38, 39]. In this experiment, it was necessary to avoid attaching a thermocouple to the tip of the drill because we are evaluating the effect of a drill point modification on thermal effects. Regarding burying a thermocouple

in the bone, a distance of 0.5 mm from the drilling hole, the closest distance measurable by the thermocouple, is considered suitable [24, 36, 40], but creating a buried hole for the thermocouple very close to the drilling hole resulted in structural vulnerability of the bone specimen. Cortical bone is dense and contains little water, so its thermo-conductive capacity is higher than in the bone marrow, with relatively rapid conduction of heat, while spongy bone has a lattice structure and contains water and lipids. So, the generation of frictional heat in the cylinder wall of spongy bone is unlikely to spread at periphery [41]. Results of various studies shown that, irrespective of drill type, more heat was generated in the superficial part of the bone (compact bone) rather than osteotomy preparation in deep part of bone (cancellous bone) [42]. As the duration of drilling is longer for the compact bone compared with the cancellous bone, the temperature increase was higher in the cortical (superficial) bone [38]. In view of the above, we decided that the most suitable method was to measure the temperature of the bone surface and drill tip during drilling using thermography. Second, the performance comparison between the commercially available and modified drills was not only done with drilling parameters during routine orthopedic surgery. Small thrust force (10 N) and low speed (300 rpm) are not chosen as bone drilling conditions for routine orthopedic surgery. Therefore, it does not negate the performance of existing commercially available drills. However, in this experiment, since commercially available medical drills could not exert good performance with minimally invasive drilling parameters, the parameters for those drilling procedures might have to be limited. Third, this experiment was performed *in vitro*, so the bone specimens were not of human origin and the drilling operation is different from actual surgical techniques. However, canine or pig bones have comparatively similar parameters such as bone mineral density and bone quality to human bones [19], and the reliability of verification results is never really suspected. Differences from the actual surgical procedure in this experiment include the use of a pig bone without blood flow and a coolant at the drilling site, such as cold water. It is expected that temperatures actually generated during bone drilling will be lower. It will also be necessary to verify thermal damage in living animals and conditions similar to actual surgical procedures. Equally important, in *in vivo* experiments, the effects of the reduction in cutting resistance and thermal exposure provided by Gekkou-modified drills on bone tissue must be histopathologically verified.

5. Conclusion

The Gekkou modifications clearly suppressed the temperature rise during drilling and increased the feed rate by several factors via lowering the cutting resistance compared with conventional drills. Drilling with Gekkou-modified drills was superior to conventional high speed drilling, and had higher performance even at conditions of low thrust and low speed. We believe that this modification reduces thermal damage to bone tissue and can lead to an improvement in clinical outcomes.

Declarations

Author contribution statement

Makoto Enokida: Conceived and designed the experiments; Performed the experiments; Analyzed and interpreted the data; Contributed reagents, materials, analysis tools or data; Wrote the paper.

Haruhisa Kanaya, Kazutake Uehara & Masaru Ueki: Performed the experiments.

Hideki Nagashima: Contributed reagents, materials, analysis tools or data.

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Competing interest statement

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Additional information

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