

# Influence of bone density and implant drill diameter on the resulting axial force and temperature development in implant burs and artificial bone: an in vitro study

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Received: 10 August 2015 / Accepted: 15 November 2015 / Published online: 20 November 2015  
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## Abstract

**Purpose** The aim of this study was to determine how the bone density affects the temperature development in artificial bone and drill.

**Methods** Ten single drills with diameters of 2.2, 2.8, 3.5, and 4.2 mm were used on four artificial bone blocks (density I–IV), with constant speed and external irrigation. Temperature measurement in blocks and drills was done by infrared camera. The resultant axial force was measured, and light microscopic examinations of the drills were performed before and after preparation.

**Results** The block density has a greater influence on resulting axial force than the drill diameter (D1=2.2 mm, 4.11 ± 0.64 N; 4.2 mm, 9.69 ± 0.78 N vs. D4=2.2 mm, 0.5 ± 0.18 N; 4.2 mm, 1.23 ± 0.08 N). For the narrowest drill, a decrease in bone density caused a significant temperature increase in the bone and drill. However, for the thickest drill, no thermal differences were found in the bone but were seen in the drill itself (D1=2.8 mm vs. D4=2.8 mm; bone  $p < 0.0001$ , drill  $p < 0.0001$ ; D1=4.2 mm vs. D4=4.2 mm; bone  $p = 0.5366$ , drill  $p = 0.0411$ ). An increase in the drill diameter in the highest bone density led to a significant thermal increase in the bone and drill. However, for the lowest bone density, thermal changes were observed only in the bone

(D1=2.8 mm vs. D1=4.2 mm; bone  $p < 0.0001$ , drill  $p < 0.0001$ ; D4=2.8 mm vs. D4=4.2 mm; bone  $p < 0.0102$ , drill  $p = 0.1784$ ).

**Conclusions** Thermal development depends on bone density with increasing density causing a temperature rise. However, this effect is reduced with increasing drill diameter. This may be important with regard to bone reactions and also in terms of tool wear.

**Keywords** Heat generation · Infrared thermography · Implant site preparation · Axial load · Drill wear

## Introduction

Many investigations focus on the heat generated during implant site preparation. Histological studies conducted by Eriksson and Albrektsson investigated the influence of temperature on the bone [1–4]. They demonstrated that a high temperature for a short duration had almost the same effect as a low temperature for a longer duration. A low temperature for a short duration reduced the bone resorption by 10 %, while a critical temperature of 47 °C for 1 min lead to irreversible damage of the bone. Therefore, low temperatures are necessary during implant site preparation in order to achieve successful osseointegration of endosseous implants. The observed thermal increase is a multifactorial, complex process that depends on various parameters. These include inter alia drill geometry, bone density, applied axial load, irrigation and rotational speed, and sharpness of the drill.

However, most of the underlying basic researches were in vitro, and the osteotomies were usually made in non-vital bone from cows and pigs or synthetic bone blocks. A current systematic review deals with those individual factors [5]. The current state of knowledge in terms of heat generation during

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the implant site preparation is that conventional drill seems to be superior to ultrasound and trephine bur. Regarding to the drill design, a higher number of cutting edges as well a minimum of lateral cutting surface would be useful for a reduction of the contact area between the drill and bone and subsequently the amount of heat production. In contrast, the material seems to be of secondary importance, while the applied load should be selected. Further, an external flushing regardless of the amount and temperature of the liquid could be sufficient, except at extremely slow drilling about 50 rpm. Then it can be apparently done even dispense without cooling.

Temperature measurement during implant bed preparation can be conducted using a thermocouple that allows direct measurement [6] or infrared thermography, which provides an indirect estimate [7]. While thermocouples detect only a single temperature point, the infrared techniques generate an overall thermal profile. In addition, the kind of bone model varies. Usually, *in vitro* studies use bovine or porcine bone models [8–12]. According to the Misch classification [13], xenogeneic bone usually has a quality of D3 or D4 [8, 14], whereas bone from the human jaws exhibits variable structure (between D1 and D4). Therefore, synthetic foam bone blocks with different densities have been described [15–17]. These blocks, based on polyurethane, have the typical quality of oral bone, allow good reproducibility, and are less susceptible to failures.

Several studies investigated the wear caused by the drill during repeated osteotomies [11, 18–20]. These studies were usually descriptive analyses using scanning electron microscopy (SEM) or mechanical investigations considering the electrical power from torque and tension of drilling. Moreover, they mainly focused on the durability of twist drills after repeated use, and its influence on the bone. Surface corrosion, degradation, and plastic deformation were observed after 50 implant bed preparations. However, there is no consensus in the available literature on temperature changes after multiple use [9, 11, 20].

Friction during drilling affects the cutting power, machining quality, and instrument life. When drill wear reaches a certain value, increasing cutting forces and vibration lead to deterioration of the surface integrity and increase in cutting temperature. In material science, different kinds of tool wear are known [21]. Abrasive wear occurs when drill material is lost by the mechanical action of hard particles present on the surface in contact with the tool. In contrast, adhesive wear is caused by the formation and fracture of welded asperity junctions between the cutting tool and workpiece. Diffusion wear occurs when atoms move from the tool material to the workpiece material because of the differences in concentration. The rate of diffusion increases exponentially with increase in temperature. The heat generated may also bring about slight oxidation, which can help isolate the cutting tool. However, at high temperatures, the soft oxide layers are formed rapidly and then chipped away easily.

Currently, there are no reported studies that have examined temperature development in the implant drills. Therefore, in the present study, we investigated the influence of bone density on the generation of heat in the bone and drills and the associated force development. The aim is to identify the possible consequences for clinical practices. These include replacement of the drill because of wear and ways to deal with the resultant axial loads.

## Materials and methods

Drilling was conducted on four different kinds of artificial polyurethane bone blocks (#1522-04, #1522-03, #1522-01, and #1522-23; Sawbones, Malmö, Sweden). These blocks have already been used successfully in other dental implant studies [22–26]. The American Society for Testing and Materials approved this material, recognized it as a standard for testing orthopedic devices and instruments, and identified it as an ideal material for comparative testing of bone screws (ASTM F-1839-08). Based on the density, the Solid Rigid Polyurethane Foam (SRPF) blocks are classified into D1=0.48 g/cc, D2=0.32 g/cc, D3=0.16 g/cc, and D4=0.08 g/cc.

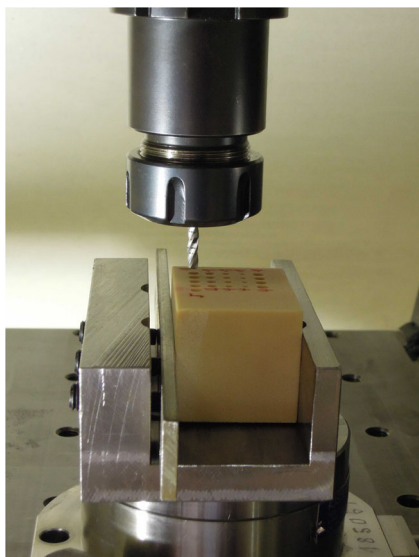
The evaluation of flank wear of the drills was performed before and after implant site preparation using a Nikon SMZ-U light microscope (Freyer Co Inc, Cincinnati, OH).

In each test block, 10 single burs were used with surgical twist drills (Institute Straumann AG, Basel, Switzerland) having diameters of 2.2, 2.8, 3.5, and 4.2 mm. For each diameter, a drilling depth of 12 mm was set. Drilling speed was approximately 1500 rpm, and external irrigation was conducted at room temperature ( $21 \pm 1$  °C) at a constant rate of 50 ml/min. The blocks were fixed in a metal container, and an actuator was moved downwards so that a gap of a few millimeters was left between the drill and block (Fig. 1). From this position, the actuator was displaced vertically at a speed of 2 mm/s, and the axial force was measured during the drilling.

Thermal images of the drill and implant site preparations were taken immediately after drilling using a 14-bit digital infrared camera (FLIR I7 PRICE BURNER, Flir Systems, Danderyd, Sweden) with a 320×240 focal plane array, 8–9 mm spectral range, 0.02 K noise equivalent temperature differences, 50 Hz sampling rate, optics, germanium lens, f 20, and f/1.5. The camera was set 50 cm away from the test block for maximum spatial resolution, and the resulting images were used to determine the temperature changes in the implant drills (Fig. 2) and artificial bone blocks (Fig. 3).

## Statistical analysis

The temperature of the bone and drill and the axial forces were described in terms of means and standard deviations (SD). The 4×4 factorial design, with the factors being bur diameter

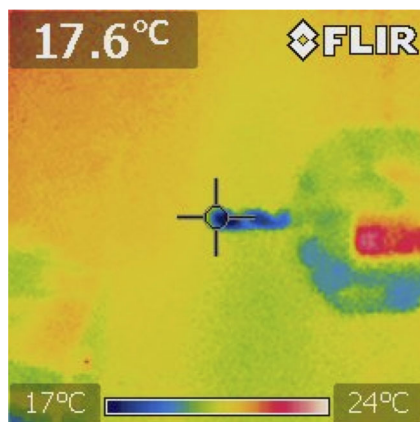


**Fig. 1** SRPF blocks fixed in a metal container, and implant drill clamped in an actuator

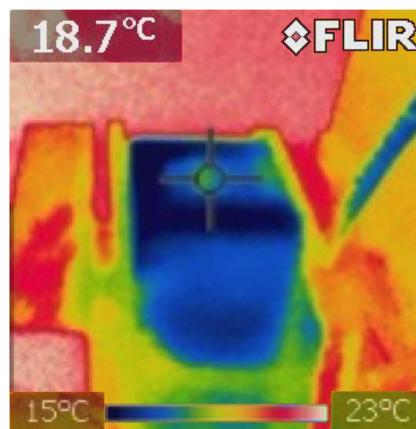
(2.2, 2.8, 3.5, and 4.2) and bone density (D1, D2, D3, and D4), was analyzed using a two-way ANOVA model, with an additional term to model the interaction between diameter and bone density. Comparisons of different diameters with the same bone density or comparison of different bone densities with the same diameter were performed by means of linear contrasts. *P* values less than or equal to 0.05 were considered to be statistically significant, and no adjustments were made to the significance level because of the explorative nature of the study. All statistical analyses were performed using SAS V9.3 Software (SAS Institute Inc., Cary, NC, USA).

## Results

The mean values for temperature increase in artificial bone blocks with four different densities and in drills with various diameters are shown in Table 1, along with the mean values



**Fig. 2** Infrared thermal image illustrating the area of maximal thermal emission of an implant drill immediately after implant site preparation



**Fig. 3** Infrared thermal image illustrating the area of maximal thermal emission of a polyurethane block immediately after implant site preparation

for axial force development. Table 2 shows the comparison of heat generation in the bone and drills, as well as the influence of the drill diameter on axial forces generated in four blocks with different densities. Table 3 shows a comparison of the average values for heat generation in bone and drills, as well as the influence of bone block density on axial forces generated using four different implant drills. The relationship between temperature development in bone and drill and the axial force is presented as a line chart in Fig. 4.

Light microscopic examination after implant site preparation showed flank wear (Fig. 5) of 52.73  $\mu\text{m}$  for the 2.2-mm drill, 16.72  $\mu\text{m}$  for the 2.8 mm drill, 17.82  $\mu\text{m}$  for the 3.5-mm drill, and 21.58  $\mu\text{m}$  for the 4.2-mm drill. Thus, deformation in the thinnest drill is more than twice that of the thickest drill.

The highest mean temperature for prepared implant site was 17.53  $^{\circ}\text{C}$  (SD=0.65), and the lowest was 14.84  $^{\circ}\text{C}$  (SD=1.78). Both were observed in D1 bone density. The highest mean temperature generated in the implant drill was 21.65  $^{\circ}\text{C}$  (SD=1.51), and the lowest was 18.32  $^{\circ}\text{C}$  (SD=1.91), also seen in D1 bone density. The highest value of axial force generated was about 9.69 N (SD=0.78) with the thickest drill of 4.2 mm in the highest bone density D1, and the lowest value was about 0.5 N (SD=0.18) with the thinnest drill of 2.2 mm in D4 bone density.

Statistically significant differences in temperature performance (Table 2) were observed between the narrow and thick drills (2.2 vs. 4.2 or 2.8 vs. 4.2 for all bone densities ( $p < 0.05$ ), except in the implant site prepared with 2.2-mm-diameter drill in D1 bone density ( $p = 0.5059$ ). Furthermore, significant differences were noticed in the D2 and D4 density artificial bone blocks, with 2.2-, 2.8-, and 3.5-mm-diameter drills ( $p < 0.05$ ). Other comparisons of temperature developments between drills with similar diameters showed no statistically significant differences or any clear patterns.

Comparison of heat generation in the drill itself showed statistically significant differences between the 2.2-mm-

**Table 1** Mean values for temperature in bone and drill as well as related axial force after the use of drills with various diameters in four different artificial bone blocks

Bone density		Drill diameter (mm)							
		2.2 (n=10)		2.8 (n=10)		3.5 (n=10)		4.2 (n=10)	
			SD		SD		SD		SD
D1	Temp. bone (°C)	17.53	0.65	14.84	1.78	17.01	0.65	17.27	0.98
	Temp. drill (°C)	19.12	0.36	18.32	1.91	20.45	0.56	21.65	1.51
	Axial force (N)	4.11	0.64	5.95	0.67	7.56	0.58	9.69	0.78
D2	Temp. bone (°C)	14.95	0.63	15.89	0.63	15.96	1.15	17.28	0.53
	Temp. drill (°C)	19.36	1.55	20.86	0.23	20.22	0.84	21.34	0.54
	Axial force (N)	1.64	0.11	2.67	0.14	3.01	0.56	3.92	0.15
D3	Temp. bone (°C)	15.72	0.25	15.04	0.63	16.39	0.6	17.18	0.99
	Temp. drill (°C)	20.48	0.47	20.54	0.34	20.57	0.42	21.35	0.21
	Axial force (N)	0.87	0.15	1.84	0.11	2.03	0.16	2.15	0.95
D4	Temp. bone (°C)	15.64	0.79	16.57	1.28	17.18	0.85	17.49	0.49
	Temp. drill (°C)	21.31	0.15	21.41	0.93	21.51	0.78	20.95	0.6
	Axial force (N)	0.5	0.18	0.92	0.07	1.04	0.09	1.23	0.08

diameter drill and the others, in bone density D1 and D2, but not in D3 and D4 (all *p* value<0.05). Furthermore, significant differences were observed when comparing the 4.2-mm-diameter drill on D1, D2, and D3 (all *p*<0.05).

Statistically significant differences in axial forces were observed only in comparisons with the 2.2-mm-diameter drill (*p*<0.05). In contrast, comparison of the larger diameters

demonstrated no differences in D3 and D4 block densities. However, D1 and D2 artificial bone exhibited statistically significant differences (*p*<0.05) between all drill diameters, except when comparing 2.8 mm with 3.5 mm diameter (*p*=0.0887).

Heat generation in the artificial bone blocks, influenced by various factors (Table 3), also showed statistically significant differences. When comparing the average temperature values

**Table 2** *P* value comparison of heat generation in bone, drill, and axial force development depending drill diameter in for different block densities

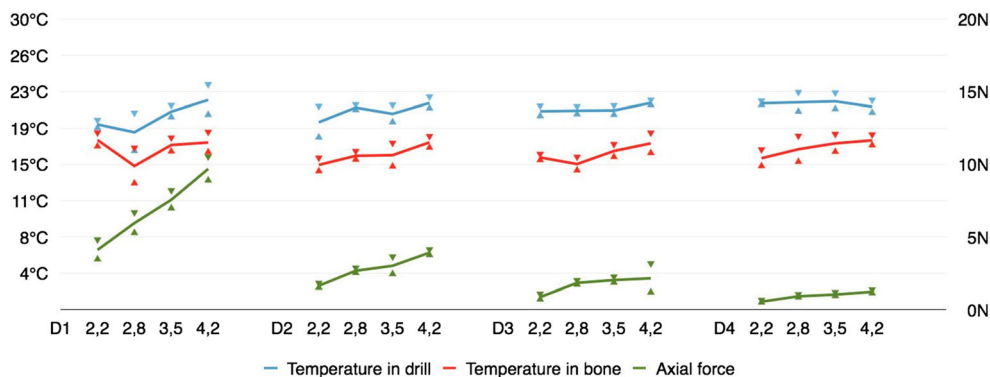
Diameter		Block quality			
		D1	D2	D3	D4
2.2 vs. 2.8	Temp. bone	<0.0001	0.0087	0.0569	0.0094
	Temp. drill	0.0197	<0.0001	0.8605	0.7696
	Axial force	<0.0001	<0.0001	<0.0001	0.1336
2.2 vs. 3.5	Temp. bone	0.1448	0.0049	0.0505	<0.0001
	Temp. drill	<0.0001	<0.0001	0.7921	0.5582
	Axial force	<0.0001	<0.0001	<0.0001	0.1127
2.2 vs. 4.2	Temp. bone	0.4653	<0.0001	<0.0001	<0.0001
	Temp. drill	<0.0001	<0.0001	0.0113	0.2922
	Axial force	<0.0001	<0.0001	<0.0001	0.0304
2.8 vs. 3.5	Temp. bone	<0.0001	0.8441	0.0002	0.0874
	Temp. drill	<0.0001	0.0617	0.9300	0.7696
	Axial force	<0.0001	0.1134	0.9618	0.8568
2.8 vs 4.2	Temp. bone	<0.0001	0.0001	<0.0001	0.0102
	Temp. drill	<0.0001	0.0213	0.0182	0.1786
	Axial force	<0.0001	<0.0001	0.8265	0.4206
3.5 vs 4.2	Temp. bone	0.4653	0.0003	0.0272	0.3841
	Temp. drill	0.0005	<0.0001	0.0230	0.1018
	Axial force	<0.0001	0.0003	<0.0001	0.5380

**Table 3** *P* value comparison of heat generation in bone, drill, and axial force development depending on artificial bone block density after using four different implant drills

Diameter		Drill diameter			
		2.2	2.8	3.5	4.2
D1 vs. D2	Temp. bone	<0.0001	0.0034	0.0034	0.9776
	Temp. drill	0.4823	<0.0001	0.5007	0.3643
	Axial force	<0.0001	<0.0001	<0.0001	<0.0001
D1 vs. D3	Temp. bone	<0.0001	0.5742	0.0824	0.8004
	Temp. drill	<0.0001	<0.0001	0.7253	0.3799
	Axial force	<0.0001	<0.0001	<0.0001	<0.0001
D1 vs. D4	Temp. bone	<0.0001	<0.0001	0.6329	0.5366
	Temp. drill	<0.0001	<0.0001	0.0021	0.0411
	Axial force	<0.0001	<0.0001	<0.0001	<0.0001
D2 vs. D3	Temp. bone	0.0313	0.0175	0.2276	0.7787
	Temp. drill	0.0012	0.3490	0.3058	0.9766
	Axial force	<0.0001	0.0003	<0.0001	<0.0001
D2 vs. D4	Temp. bone	0.0534	0.0569	0.0007	0.5553
	Temp. drill	<0.0001	0.1080	0.0002	0.2539
	Axial force	<0.0001	<0.0001	<0.0001	<0.0001
D3 vs. D4	Temp. bone	0.8221	<0.0001	0.0272	0.3841
	Temp. drill	0.0156	0.0113	0.0062	0.2420
	Axial force	0.0958	<0.0001	<0.0001	<0.0001



**Fig. 4** The interaction between temperature development in bone and drill related to the axial force



for the 2.2-mm-diameter drill, significant differences were observed between the highest bone density (D1) and decreasing bone densities (D2, D3, and D4) ( $p < 0.05$ ), but not for the other density comparisons using this diameter. In contrast, no differences were found for all comparisons of bone density using the 4.2-mm bur.

The thermal development in the drills showed no asymmetrally in the drill flanges and behaved similar to that in the bone when using 2.8-, 3.5-, and 4.2-mm-diameter drill. With the exception of the comparison between D1 and D2, significant temperature differences were observed between all bone densities, using the 2.2-mm drill ( $p < 0.005$ ). In contrast, no statistically significant differences in heat generation were found after using the 4.2-mm drill. With regard to other possible comparisons, no clear patterns of behavior were observed.

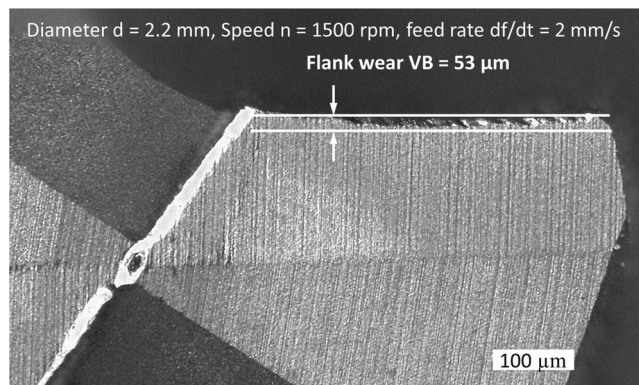
The axial force development depending on artificial bone block density showed statistically significant differences for all comparisons ( $p < 0.001$ ), except between D3 and D4 block densities with the 2.2-mm drill ( $p = 0.0671$ ).

**Discussion**

The comparison of the information available in the current literature on heat development during implant site preparation

is difficult because of the differences in the study models used. Usually, two different methods are used for recording real-time temperature rises. The thermocouple allows direct measurement [8, 10, 12, 15, 18, 27, 28], while infrared thermography provides an indirect estimate [12, 17, 29]. Though the thermocouple technology is the most common way to detect thermal increases during implant site preparation, current studies are not uniform in design. These differences lie in the distance to the final bone cut (from 0.5 to 2 mm), the element orientations (from mono to tripod configuration), and depths and numbers in the vertical dimension (mono- and multichannel). In addition, this technique allows detection of spot temperatures only, and therefore, an overall thermal profile is not possible. This problem does not exist with infrared technology, which is described as being more accurate and having a lower probability of error [29]. However, in this investigation, a thermal measurement during the drilling is not possible because of the necessary direct field of view for the infrared camera. Using transparent artificial bone could circumvent this. But to our knowledge, no blocks with corresponding bone qualities are available at present. Furthermore, the temperature development in the artificial bones as well as the drill itself would overlap itself. This would make an accurate measurement difficult and led to a direct measurement after the drilling.

There is no standardized bone model for investigation on implant site osteotomy. Various osseous models based on cadaveric tissues of bovine or porcine bone blocks have been used [8, 10, 12, 18–20, 27–29], while others used synthetic blocks [15–17, 30]. Strbac et al. were able to show that using a standardized synthetic bone model is a positive development in materials research as it allows standardization and reproducibility of test results [16]. They used an artificially manufactured bone that provides equal vertical and horizontal parameters and corresponds to clinical conditions in human bone (type 2 according to Lekholm and Zarb classification) [31]. They reported that this synthetic bone provides analog thermal conductivity to the human bone (0.3–0.4 W/m/K), thereby making comparison of temperature changes possible [32]. With this model, a standardization of the output parameters is possible.



**Fig. 5** Light microscopic examination after implant site preparation showed flank wear for 2.2-mm drill, using a Nikon SMZ-U light microscope (Freyer Co Inc, Cincinnati, OH)

Some studies investigated the temperature increase with different loads during implant site drilling [10, 28, 33, 34]. Rashad et al. did not find any differences in the resulting temperature development with various axial loads (5, 8, 15, and 20 N) during conventional drilling, for cortical and cancellous bone [10]. Likewise, Stelzle et al. were interested in the effect of axial pressure on the hard tissue using various loads between 0 to 1000 g, during implant site preparation [28]. The maximum temperature increase was about 45.5 °C at a load of 500 g, followed by a thermal decrease with higher vertical loads. The additional histomorphometric examinations demonstrated maximum alterations of 166.0 µm with the same load. In contrast, our results suggest a relationship between the axial load and heat generation in artificial bone blocks and implant drills. It appears that increasing pressure on the drill leads to an increase in temperature. However, in this investigation, the thermal increase was affected by multiple factors, but not by axial load as a constant vertical speed had been adopted. Therefore, a possible explanation may be the different bone blocks and the various diameters of the drills. While increasing block densities lead to an increase in drill resistance, the increase in drill diameter in combination with constant drilling speed led to a higher cutting speed. Our *p* value comparison of axial force development is more sensitive to block density than drill diameter. In this context, the temperature development in the artificial bone or the implant drills did not correlate with the resultant axial force.

Furthermore, our study showed an influence of bone density and drill diameter on temperature development. Significant changes in temperature development were observed when comparing the smallest and largest drills. However, it was shown that the effect of the drill diameter was more evident with increasing density of the artificial bone. It is probable that the thermal increase was caused by a higher cutting speed, resulting from increasing diameter with the same drilling speed. Therefore, with increasing diameter, an increase in temperature may be expected, except with the narrow drill (2.2 mm diameter) in very hard bone (Type D1). Thus, our findings correspond to the results reported by Strbac and colleagues [16, 30]. Using a real-time thermocouple model, they recognized statistically significant higher temperatures with a 2-mm drill when compared to 3.5-, 4.3-, and 5-mm-deep conical drills, with and without continuous cooling.

According to the classification by Misch [13] or Lekholm and Zarb [31], the human jaws exhibit different bone qualities. While D1 bone density is present in the anterior mandible, D2 and D3 bone densities can usually be found in the anterior to posterior mandible and maxilla. D4 bone density is typically found in the posterior maxilla, like the tuberosity region. The bone qualities I to IV, or D1 to D4, are characterized by increasing proportions of cancellous bone, thus decreasing density. In this context, the thermal conductivity varies between cortical and cancellous bone structures. A different vascular

penetration rate (cancellous bone, 0.5 mm per day; cortical bone, 0.05 mm per day) probably accounts for this [35]. Histological studies by Stelzle et al. investigated the effects of osteotomy in both bone structures [28]. They found the highest thermal effects in the cortical areas. Until now, thermal effect depending on various bone densities has not been investigated. Our study indicates that significant differences in temperature development were found only when using the 2.2- and 2.8-mm burs. With increasing diameter, 3.5 to 4.2 mm, differences in thermal effect between different bone qualities disappeared. This demonstrates that bone density has less influence on the temperature development than drill diameter.

From the manufacturing technology, it is known that a solid-state diffusion occurs when atoms move from a region of high atomic concentration to one of low concentration. This process causes a weakening of the surface structure of the tool and is called diffusion wear [36]. This depends on temperature, as the diffusion rate increases exponentially with increase in temperature and atoms may move from tool material to the work material. Therefore, the cutting edge of the drill will be increasingly softer and deform plastically. Various studies have analyzed cratering and tool life in terms of cutting conditions, friction characteristics, and material properties. Optimal conditions with respect to tool life can be found with decrease in temperature [37]. The wear of implant drills is analyzed using scanning electron microscopy [11, 20]. Usually, the aim of these investigations is to recognize the durability of drills after multiple uses and their influence on the bone. The sharpness of a drill is related to the number of uses, applied load, sterilization techniques, bone density, construction, and material. It is known that a high correlation between the amount of damage and number of uses exists. After 50 implant site drillings, the surface showed corrosion, degradation, and plastic deformation. While some authors observed no significant temperature differences after repeated preparations [9, 11], other investigations reported an increase in temperature with rising number of bur use [20]. Regardless of drill wear, in this study, the temperature development in the drills behaved similar to that in the bone. However, the temperatures are always higher than those in artificial bone. Comparison of thermal development in the narrowest drills with those with increasing diameter demonstrated significantly higher values during drilling in high (D1 and D2) density blocks only. This proves that with decreasing block density, the diameter of the drill had no effect on heat generation in the tool itself. In this context, it must be assumed that differences in tool wear can only be found between thin and thick drills in implant site preparations with high bone density. Our light microscopic examination after implant site-drilling showed maximum flank wear for the 2.2-mm drill. The other drills demonstrated much lower deformations, which increased with increasing diameter. Thus, except for the thinnest drill, a relationship

seems to exist between drill temperature and wear. However, more studies with a higher sample size are needed to confirm this statement.

## Conclusions

Bearing in mind the limitations of this experimental in vitro study using synthetic bone blocks, it can be concluded that increasing the diameter of the drill for a particularly bone density will result in an increase in the axial force generated. Temperature development in bone and drill behaves similarly, but is generally higher in drills. Therefore, thermal increase influenced by drill diameter is higher than that caused by bone density, but with decreasing block densities the diameter of the drill has no effect on heat generation in the tool itself. The critical temperature level of 47 °C was not exceeded at any time. The strongest wear can be found in the smallest drill, even though it exhibits the slightest cutting speed. Presumably, differences in tool wear for drills with different diameters can be expected after implant site preparation in artificial bone with high density. Further in vivo studies are required to determine if these results can be applied to humans.

## Compliance with ethical standards

**Conflict of interest** The authors declare that they have no competing interests.

**Disclosure** The authors do not have any financial interests or commercial associations to disclose.

**Ethical approval** This article does not contain any studies with human participants or animals performed by any of the authors.

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