Micromorphometric analysis of bone blocks harvested with eight different ultrasonic and sonic devices for osseous surgery

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ABSTRACT

Objectives: The aim of this study was to analyse in vitro the main features of osteotomies performed by means of different ultrasonic and sonic systems for bone surgery.

Materials and methods: Six ultrasonic and two sonic devices for osseous surgery were evaluated during block harvesting on bovine bone. After measuring cutting speed, images of the blocks were acquired by light stereo-microscope and E-SEM, in order to measure the osteotomy thickness and to evaluate the presence of intra-trabecular bone debris and signs of thermal injuries on the bone. Roughness evaluation was performed using a profilometer.

Results: All the ultrasonic instruments required a shorter time than sonic systems to perform the block harvesting (p < 0.05). Piezomed was found to be the most efficient in terms of cutting speed (20.5 mm²/min), even if not significantly different from most of the devices here tested (p > 0.05). K-Bisonic and Variosurg 3 showed the smallest percentage variance between tip thickness and osteotomy width. Intra-trabecular debris was found to occur in inverse proportion with the width of the osteotomy: the tighter the track, the higher the amount of debris. Sonicflex Bone, Piezotome 2 and Sonosurgery showed almost no signs of thermal injuries on the osteotomised surfaces.

Conclusions: No single ultrasonic or sonic device combined all the best features of speed, precision and bone micro-architecture preservation.

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1. Introduction

The piezoelectric osteotomy is the result of bone micronisation produced by mechanical shock waves with a linear vibration ranging from 24 to 36 kHz and with an amplitude varying from 20 to 200 μm: these properties produce a peculiar cutting action on hard tissues, which was extensively studied in the last decade (Vercellotti, 2000; Eggers et al., 2004; Stübinger et al., 2005; Cardoni et al., 2006; Beziet et al., 2007; Nordera et al., 2007). Main features of ultrasonic bone surgery are represented by the micrometric cut (leading to a precise and controllable surgical action) (Vercellotti, 2004; Alam et al., 2013), the selective activity on the mineralised tissues (Schaeren et al., 2008), the cavitation effect (Walmsley et al., 1990), and the positive influence of the ultrasonic cut on bone healing if compared to rotary instruments (Preti et al., 2007). Many clinical applications of piezoelectric bone cutting were described both in oral surgery (e.g., maxillary sinus floor elevation (Vercellotti et al., 2001)), ridge expansion (Anitua et al., 2013), bone block harvesting (Stübinger et al., 2008), tooth extraction (Rullo et al., 2013), implant site preparation (Stacchi et al., 2013) and in other surgical fields (maxillofacial surgery, otorhinolaryngology, orthopaedics, neurosurgery).

Recently, the use of air-driven sonic osteotomes with a vibration ranging from 3 to 6 kHz and an amplitude varying from 200 to 300 μm has been proposed for applications in oral surgery and reported in some clinical and experimental studies (Agabiti, 2011; Papadimitriou et al., 2012; Viganò et al., 2015). Sonic tips rotate with a circular tapping motion, and are oriented by the friction into the osteotomic line: inserts are active on all sides, permitting work in any direction without changing the position of the handpiece.

Nowadays, the number of sonic and ultrasonic osteotomes available on the market had remarkably increased. In vitro and animal studies (Maurer et al., 2008; Hollstein et al., 2012; Rashad et al., 2013) demonstrated differences in the micromorphology of
osteotomised bone surfaces between rotary and oscillating instruments, ultrasonic osteotomes, and among some piezoelectric devices themselves. Bone cut with microvibrations preserves the osseous architecture, especially the integrity of the trabeculae of the cancellous bone which, on the contrary, loses its typical structure after conventional osteotomies performed with burs or saws (Maurer et al., 2008). In these cases, the cancellous spaces are condensed with osseous debris, which represents a mechanical obstacle for the centrifugal blood supply (Schweiberer et al., 1974; Simonetti et al., 2013). Many authors underline that the preservation of the cancellous bone structure enhances the quality and the speed of the bone healing process, due to the high osteogenic potential of the spongy bone (Soldner and Herr, 2001; Rundle et al., 2006).

Hence, the objectives of this in vitro study were to analyse and compare the bone cutting performance of eight different sonic and ultrasonic devices when harvesting bovine bone blocks, in terms of cutting speed, surgical precision and micromorphology of the osteotomised bone surfaces.

2. Materials and methods

2.1. Investigational devices

Between February and April 2014, thirteen manufacturers and distributors of sonic and ultrasonic devices for osseous surgery were invited to join this study. An e-mail containing the study protocol was followed by direct phone calls from one investigator (CS), in order to thoroughly illustrate the project and its objectives to the invited companies. Eight manufacturers agreed to participate (CS), in order to compare the bone cutting performance of eight different sonic and ultrasonic devices when harvesting bovine bone blocks, in terms of cutting speed, surgical precision and micromorphology of the osteotomised bone surfaces.

Table 1

<table>
<thead>
<tr>
<th>Invited manufacturers and distributors (bold type is used for the companies who agreed to participate in the study).</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Ultrasonic devices</strong></td>
</tr>
<tr>
<td>K-Bisonic (Kirmed, Italy); MiniUNIKO PZ, Mariotti, Forlì, Italy</td>
</tr>
<tr>
<td>Piezomod, W&amp;H, Bürmoos, Austria</td>
</tr>
<tr>
<td>Piezon Master Surgery, EMS, Nyon, Switzerland</td>
</tr>
<tr>
<td>Piezotomie Touch (Mectron, Italy); Piezosurgery Touch (Mectron, Italy); Piezotome 2 (Acteon Satelec, France); Surgysonic Moto (Esacrom, Italy); Variosurg 3 (NSK, Japan) and two sonic systems Sonicflex Bone (Kavo, Germany); Sonosurgery (Komet/TKD, Germany/Italy)</td>
</tr>
<tr>
<td><strong>Sonic devices</strong></td>
</tr>
<tr>
<td>SonicBone, Zelching, Germany</td>
</tr>
<tr>
<td>Komet (Komet/TKD, Germany/Italy)</td>
</tr>
</tbody>
</table>

Table 1 Invited manufacturer and distributors (bold type is used for the companies who agreed to participate in the study).

Fig. 1. Tips chosen by the companies to perform the test: left to right Piezosurgery Touch (Mectron); Variosurg 3 (NSK); Sonosurgery (Komet/TKD); Sonicflex Bone (Kavo); K-Bisonic (Kirmed); Piezotome 2 (Acteon Satelec); Piezomed (W&H); Surgysonic Moto (Esacrom).

2.2. Experimental phase

Three operators with different levels of expertise were selected: operator A (CS) was an oral surgeon with more than ten years of routine practice in ultrasonic bone surgery, operator B (MF) was an expert maxillofacial surgeon who only occasionally used piezoelectric devices and operator C (FB) was a resident in Oral Surgery, with a still limited practice both in conventional and in ultrasonic osseous surgery. A fourth operator (IA – see acknowledgments), expert in sonic bone surgery, was recruited to test the two sonic devices as operator A: in these two experimental sessions CS worked as operator B and FB as operator C.

Bone-cutting performance of ultrasonic and sonic devices was evaluated during the harvesting of square shaped corticocancellous bone blocks (15 mm side length, 10 mm depth, at least 2 mm of cortical bone) from fresh bovine ribs, cleared of soft tissues, at room temperature. Block perimeter was previously marked with a pencil on the surface of the rib, by using a titanium template. Each operator (A,B,C) harvested one bone block with each investigated surgical device: all osteotomies were performed following the manufacturer’s instructions, and conducted under irrigation with cooled 0.9% sodium chloride solution. Tests were performed in the presence of a representative for each participating company, who installed and checked the device with the selected tip, adjusted power settings and irrigation, and assisted in the experimental phase.

2.3. Cutting speed and osteotomy thickness measurement

Time required for bone block harvesting was recorded using a digital chronograph, from the beginning to the end of the programmed osteotomies. A time limit of 20 min was fixed to complete the bone cutting procedure and to be included in the subsequent evaluation. The cutting speed (mm²/min) was obtained dividing the area of the osteotomised cortical bone by the time requested for
the cutting procedure. The area (mm²) of the cortical bone was obtained multiplying the length of the block side by the average thickness of the cortical bone.

Before removing the osteotomised bone block from the whole bone sample, an image for each side of the block was collected at 2× magnification under a light stereo-microscope (MZ-16, Leica Microsystems, Heerbrugg, Switzerland) in order to evaluate the osteotomy thickness by means of an image analysis software (Image ProPlus 6.2, Media Cybernetics, Marlow, UK). A graduated grid was superimposed to the picture (Fig. 2) in order to guide the correct spacing among the evaluating segments.

2.4. Roughness evaluation

Average micro-roughness (Ra) of the osteotomised surface of the cortical bone was recorded using a profilometer (Talysurf CLI 1000, Taylor Hobson, Chicago, USA), with an inductive gauge (lateral resolution 50 nm – vertical resolution 10 nm), oriented perpendicularly to the sample. Scanning analyses were performed with an acquisition speed of 50 µm/s and 24 measurements were taken for each sample (single measurement length = 1.5 mm; interval between two measurements = 2 mm). All parameters were standardized to an 80 µm cut-off filter.

2.5. Osteotomic surface micromorphological evaluation

Blocks were gently removed from the ribs and images of the entire osteotomical surfaces were acquired under a light stereo-microscope at 20× magnification (MZ-16, Leica Microsystems, Heerbrugg, Switzerland). Four images per side of each block were acquired. Two calibrated observers (FB and CON) independently analysed the images on a high definition 24-inch screen, recording
the presence of eventual signs of thermal injuries on the cut surface, which were evaluated with a score equal to 0 = absent, 1 = light, 2 = moderate and 3 = high (Fig. 3a). The presence of metal debris dispersed by the insert was also recorded (Fig. 3b). Intra-examiner reliability was assessed with Cohen’s K test (Landis and Koch, 1977).

Finally, bone specimens were fixed in 10% phosphate buffered formalin for one month at 4 °C and subsequently dehydrated in increasing concentrations of alcohol (50–100% v/v). Samples were then air-dried at room temperature for 24 h and subsequently mounted on aluminium stubs covered with two-sided conductive carbon adhesive tape. Samples were then analysed by means of a scanning electron microscope (Quanta250 SEM, FEI, Oregon, USA) operating in environmental conditions (E-SEM; p = 100 Pa). The working distance was adjusted in order to obtain the suitable magnification and the accelerating voltage was set to 30 kV.

Two calibrated observers (FB and CON) independently analysed the images on a high definition 24-inch screen, recording the presence intra-trabecular debris. Bone debris was scored on a 0–4 scale (0 = no debris; 1 = debris<25%; 2 = 25% ≤ debris<50%; 3 = 50% ≤ debris<75%; 4 = debris>75%) (Fig. 4). Intra-examiner reliability was assessed with Cohen’s K test (Landis and Koch, 1977).

### 2.6. Statistical analysis

Normality of the data distribution and homoscedasticity assumptions were assessed respectively by means of Kolmogorov–Smirnov and Levene test. Since the data did not present a normal distribution, the statistical significance of the difference for the variables Cutting Speed, Average Roughness (Ra) and Intra-trabecular debris scores among the Devices groups was assessed by means of Kruskal–Wallis non-parametric test. When significant interactions were seen, a Bonferroni corrected Mann–Whitney U-Test was performed for pairwise comparison. Statistical significance was pre-set at α = 0.05.

### 3. Results

#### 3.1. Cutting speed

The value of the median for the cutting speed of the tested devices varied from 5.6 to 20.5 mm²/min (Fig. 5). The results, expressed as median [25th percentile; 75th percentile] in mm²/min, of the single devices were the following: Piezosurgery Touch 9.7 [9.0; 12.6], Variosurg 3 7.3 [6.5; 9.8], Sonosurgery 5.6 [4.8; 5.8], K-Bisonic 7.7 [7.3;

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**Fig. 3.** Light stereo-microscope images of samples showing the presence of thermal injuries (a) and metal debris (b) on the osteotomised bone surface.

**Fig. 4.** Environmental scanning electron microscope images showing two surfaces with different amounts of intra-trabecular bone debris (grade 1 on the left — grade 4 on the right).
3.2. Track thickness

The correlation between insert thickness and track thickness was calculated using Spearman correlation test (the thickness of every tip is reported in Fig. 7). A direct correlation was demonstrated between the thickness of the insert with the average of the track thickness obtained by the three surgeons ($p = 0.428$). Track thickness was calculated as the average ± standard deviation of the three tracks obtained by each surgeon (Fig. 7): Piezosurgery Touch (0.594 ± 0.106), Variosurg 3 (0.868 ± 0.132), Sonosurgery (0.625 ± 0.221), Sonicflex Bone (0.953 ± 0.147), K-Bisonic (1.163 ± 0.179), Piezotome 2 (0.900 ± 0.117), Piezomed (0.643 ± 0.161), Surgynsonic Moto (0.715 ± 0.079).

The thickness of the osteotomized track results in an inverse proportion with the tip thickness: narrow inserts produced relatively wide osteotomies.

3.3. Roughness assay

The micro-roughness ($R_a$) median of the osteotomized cortical bone varied from 0.45 to 0.88 μm among the eight devices (Fig. 8). $R_a$ values, expressed as median [25th percentile; 75th percentile] in μm, recorded for each system were the following: Piezosurgery Touch 0.54 [0.43; 0.74], Variosurg 3 0.45 [0.35; 0.55], Sonosurgery 0.49 [0.33; 0.69], Sonicflex Bone 0.88 [0.67; 1.18], K-Bisonic 0.59 [0.44; 0.79], Piezotome 2 0.62 [0.48; 0.82], Piezomed 0.51 [0.40; 0.62], Surgynsonic Moto 0.53 [0.37; 0.69]. A graphical description of the results, associated with statistically significant differences is reported in Fig. 8.

3.4. Micro-morphological evaluation of the osteotomic surface

Micro-morphological analysis of the osteotomized bone surface was performed by two independent calibrated observers ($K = 0.84$). The trabecular micro-architecture of the cancellous bone was preserved in all the samples obtained by means of the devices here investigated.

Piezosurgery Touch, Sonosurgery, Piezomed, and Surgynsonic Moto showed the highest intra-trabecular presence of bone debris (>75%), covering the majority of the surfaces, while Variosurg 3 and Piezotome 2 the lowest, as expressed in Fig. 9. In all the examined samples, there was a direct correlation between the thickness of the osteotomized track and the cleansing of the bone surface: narrow osteotomies appeared always associated with the presence of large amounts of intra-trabecular debris.
Fig. 6. Cutting speed (mm²/min) of the three operators with all of the devices: data are presented as box plots (0–25th; 25th–50th; 50th–75th; 75th–100th percentiles). Operator legend: A – oral surgeon with more than ten years of routinely practice in ultrasonic bone surgery, B – expert maxillofacial surgeon who only occasionally used piezoelectric devices and C – resident in Oral Surgery. Kruskal–Wallis test did not exhibit significant differences (p = 0.462).

Fig. 7. White filled columns show the mean osteotomic track thickness (mm) for each device; striped columns represent the tip thickness. \( \Delta \) represents the percentage track enlargement calculated as the ratio of the difference between the track thickness and the tip thickness, and the tip thickness itself. Devices legend: A – Piezosurgery Touch (Mectron); B – Variosurg 3 (NSK); C – Sonosurgery (Komet/TKD); D – Sonicflex Bone (Kavo); E – K-Bisonic (Kimed); F – Piezotome 2 (Acteon Satelec); G – Piezomed (W&H); H – Surgysonic Moto (Esacrom).
Traces of metal debris on the bone surface were present in samples obtained by K-Bisonic device (26.3% of the recorded images). Sonosurgery, Sonicflex Bone and Piezotome 2 showed almost no signs of thermal injuries on the osteotomised surfaces (0–3.8%), while Piezomed and Surgysonic Moto exhibited more than half of the images (59.1–60.4%) with thermal injuries of various degrees. Complete results are reported in Table 3.

### 4. Discussion

Ultrasonic and sonic osteotomes demonstrated some advantages in comparison with rotary and oscillating conventional

![Fig. 8. Micro-roughness ($R_a$) values ($\mu m$) for the eight devices of the investigation; data are presented as box plots (0–25th; 25th–50th; 50th–75th; 75th–100th percentiles). Groups identified by different lowercase Latin letters are significantly different ($p < 0.05$). Statistical differences were assessed by means of Mann–Whitney U-test; $p < 0.05$.](image)

Fig. 8. Micro-roughness ($R_a$) values ($\mu m$) for the eight devices of the investigation; data are presented as box plots (0–25th; 25th–50th; 50th–75th; 75th–100th percentiles). Groups identified by different lowercase Latin letters are significantly different ($p < 0.05$). Statistical differences were assessed by means of Mann–Whitney U-test; $p < 0.05$.

![Fig. 9. Evaluation of intra-trabecular bone debris presence for each device.](image)

Fig. 9. Evaluation of intra-trabecular bone debris presence for each device.

<table>
<thead>
<tr>
<th>Thermal injuries</th>
<th>Tag</th>
<th>Device</th>
<th>Absent</th>
<th>Light</th>
<th>Moderate</th>
<th>High</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>A</td>
<td>Piezosurgery Touch</td>
<td>81.6</td>
<td>16.3</td>
<td>2.1</td>
<td>0.0</td>
</tr>
<tr>
<td></td>
<td>B</td>
<td>Variosurg 3</td>
<td>77.0</td>
<td>16.7</td>
<td>4.2</td>
<td>2.1</td>
</tr>
<tr>
<td></td>
<td>C</td>
<td>Sonosurgery</td>
<td>96.2</td>
<td>3.8</td>
<td>0.0</td>
<td>0.0</td>
</tr>
<tr>
<td></td>
<td>D</td>
<td>Sonicflex Bone</td>
<td>100.0</td>
<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
</tr>
<tr>
<td></td>
<td>E</td>
<td>K-Bisonic</td>
<td>62.4</td>
<td>29.2</td>
<td>6.3</td>
<td>2.1</td>
</tr>
<tr>
<td></td>
<td>F</td>
<td>Piezotome 2</td>
<td>97.9</td>
<td>2.1</td>
<td>0.0</td>
<td>0.0</td>
</tr>
<tr>
<td></td>
<td>G</td>
<td>Piezomed</td>
<td>39.6</td>
<td>16.7</td>
<td>4.2</td>
<td>39.5</td>
</tr>
<tr>
<td></td>
<td>H</td>
<td>Surgysonic Moto</td>
<td>40.9</td>
<td>50.0</td>
<td>6.8</td>
<td>2.3</td>
</tr>
</tbody>
</table>

Table 3

Presence of thermal injuries expressed in percentage for each device.

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instruments: they are mainly represented by a more precise and controllable osteotomy (Maurer et al., 2008; Parmar et al., 2011; Heinemann et al., 2012; Schmidt et al., 2013), selective cutting action on mineralized tissues (Vercellotti, 2004; Schaeren et al., 2008) and a cleansing action over the cut surface due to the cavitation effect generated by ultrasonic or sonic waves in the cooling fluid (Maurer et al., 2007; Simonetti et al., 2013).

These clinical benefits led to a rapid diffusion of ultrasonic and sonic instruments in many different fields of osseous surgery, with a remarkable increase in the number of devices available on the market. The aim of this investigation was the analysis of the osteotomies performed with eight different systems for bone surgery. Devices were evaluated in terms of speed of cutting, precision and micromorphometric characteristics of the osteotomised bone, within the limitations of the low statistical power of this trial.

The test selected was the harvesting of bone blocks from fresh bovine ribs (with a minimum of 2 mm of cortical bone), in order to simulate the heaviest working conditions in oral surgery (e.g., blocks collected from the ramus or the chin). Bovine bone is commonly used as a model in biomechanics because its cortical thickness and cancellous density are similar to human bone (Unger et al., 2010).

Cutting speed of ultrasonic and sonic devices is related to multiple factors, including tip shape, vibration velocity, applied load, tuned frequency and coupling contact conditions (O’Daly et al., 2008). Furthermore, in our study, the influence of the operator must be considered: even if the human factor can be regarded as a limitation in terms of repeatability, we preferred to conduct this trial trying to simulate the clinical reality as much as possible.

In our investigation, all of the ultrasonic instruments required a shorter time than the sonic ones to perform the block harvesting. Differences of cortical thickness among the blocks were considered, and cutting speed value was weighted for each sample to standardise the evaluation. Piezomed was the more efficient device in terms of cutting speed, whilst the statistical analysis did not show significant differences with almost all the other ultrasonic systems. Between the two sonic instruments here tested, only Sonosurgery allowed the three operators to complete the block collection within the threshold time.

The influence of the operator on the cutting speed was not an influencing factor: different levels of expertise were not correlated with significantly different cutting speed in performing osteotomies. However, it is interesting to note how the unexperienced operator (C) had faster results, even if not significantly, than the expert surgeon not accustomed to ultrasonic devices (B): the habit of using rotary instruments requiring more pressure on the hand-piece to perform an efficient cutting action could be a key to explaining this finding.

Osteotomic track thickness was found to be correlated to the tip thickness: however, narrow inserts produced relatively wide osteotomies, with a greater mean enlargement of the track compared to thicker inserts. Hence, precision of cut seemed generally influenced by insert thickness: extremely thin tips (e.g., 0.25 mm Sonosurgery and Piezomed) produced an osteotomic track even wider than thicker inserts (e.g., 0.35 mm Piezosurgery Touch).

Osteotomies performed with all the eight ultrasonic and sonic devices showed a smooth cortical surface (Ra < 1 μm), even if statistically significant differences among devices were detected, and preserved the trabecular micro-architecture of the cancellous bone. Bone microstructure integrity may significantly reduce the presence of pro-inflammatory cytokines and growth factors involved in bone healing (Soldner and Herr, 2001): even if inflammation is always the necessary basis for tissue repair, an excessive and long lasting presence of cytokines could retard the whole process (Gerstenfeld et al., 2003; Rundle et al., 2006). Hence, the low grade of inflammation following ultrasonic osteotomies could contribute to the enhanced bone healing response observed in periodontal and implant piezosurgery (Vercellotti et al., 2005; Preti et al., 2007; Stacchi et al., 2013).

Bone debris deriving from the osteotomy and condensed into the trabecular spaces of the spongious bone is common evidence after conventional osteotomies with burrs and saws (Maurer et al., 2007; Rashad et al., 2013): blood perfusion and cells migration from the marrow spaces might be delayed by this mechanical obstacle (Schweiberer et al., 1974; Simonetti et al., 2013). Instead, due to the cavitation effect, sonic and ultrasonic instruments are reported to leave a cleaner osteotomie surface, avoiding closure of marrow spaces and vascular canals (Simonetti et al., 2013; Rashad et al., 2013). The cavitation phenomenon creates a virtually bloodless surgical site that makes visibility in the working area much clearer than by using conventional bone cutting instruments: this factor helps the operator in conducting the entire procedure in optimal conditions of surgical control. Cavitation elicits also an anti-bacterial action, by fragmenting the cellular membranes of bacteria (Arrojo et al., 2008) and, furthermore, produces a cleansing action on the osteotomie surface. In this study, the presence of bone debris was found to be strictly related to the thickness of the osteotomic line: the cleansing efficacy of the cavitation effect appeared efficient in wider osteotomies. In fact, Variosurg 3, Sonicflex Bone, K-Bisonic and Piezotome 2, which showed the cleanest osteotomie surfaces, showed at the same time the widest osteotomic tracks.

Both sonic instruments showed almost no signs of thermal injuries on the osteotomised surfaces: this is in accordance with data in the literature describing sonic osteotomies as safe in terms of heat generation (Heinemann et al., 2012; Rashad et al., 2015). Signs of thermal injuries at various levels were observed in samples from ultrasonic osteotomies: among them Piezotome 2, Piezosurgery Touch and Variosurg 3 were found to be the safest devices. However, especially when using ultrasonic devices and inserts with high cutting speed, a careful loading distribution on the handpiec and copious irrigation with cooled saline solution are crucial factors to prevent unwanted excessive heat generation.

Traces of metal deposits were detected by the light microscope only in samples from K-Bisonic (26%), likely as the result of insert attrition: this is not uncommon in the literature (Rashad et al., 2013), and also could be explained considering that this tip is the only one made of titanium alloy.

5. Conclusions

From the results of the present investigation, ultrasonic devices enabled especially fast and powerful cutting compared to the sonic systems, which seemed to not be the first option for thick cortical bone or extensive osteotomies. However, no single ultrasonic or sonic device combined all the best features of velocity, precision and bone micro-architecture preservation: further evaluations are necessary to fully elucidate removal mechanisms and possible damage on hard and soft tissues, providing indications for future technological improvements in order to optimize performance and safety during daily clinical applications.

Conflict of interest

The authors declare not to have any conflict of interest related to this study.

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