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ORIGINAL PAPER

Healing of osteotomy sites applying either piezosurgery or two conventional saw blades: a pilot study in rabbits

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Abstract
Purpose The purpose of this study was to compare bone healing of experimental osteotomies applying either piezosurgery or two different oscillating saw blades in a rabbit model.
Methods The 16 rabbits were randomly assigned into four groups to comply with observation periods of one, two, three and five weeks. In all animals, four osteotomy lines were performed on the left and right nasal bone using a conventional saw blade, a novel saw blade and piezosurgery.
Results All three osteotomy techniques revealed an advanced gap healing starting after one week. The most pronounced new bone formation took place between two and three weeks, whereby piezoelectric surgery revealed a tendency to faster bone formation and remodelling. Yet, there were no significant differences between the three modalities.
Conclusions The use of a novel as well as the piezoelectric bone-cutting instrument revealed advanced bone healing with a favourable surgical performance compared to a traditional saw.

Introduction

In orthopaedic and aesthetic and reconstructive surgery, there is an increasing demand for reliable bone cutting instruments to allow osteotomies of thin and fragile bony segments, which are otherwise prone to fracture [1, 2]. A precise and safe bone cutting technique is of the utmost importance for the preservation of vital and delicate bony structures and protection of adjacent soft tissues [3]. Therefore, recent trends have aimed at the development of novel cutting instruments to improve surgical performance and manoeuverability [4, 5].

According to Giraud et al. [6], osteotomies can be classified using four different criteria: (1) end result, (2) type, (3) approach and (4) incision site.

The end result is defined as the reason for the osteotomy, the type of osteotomy corresponds to the design of the cut, the approach is either subperiosteal or extraperiosteal and finally, the incision site is specified as epiphyseal, metaphyseal or diaphyseal. Independently of chosen powered osteotomy method, either rotary or oscillating, every individual technique should fulfill the basic requirements of a satisfying cutting speed and manual accuracy. Even though most current powered bone cutting instruments allow a direct and straightforward osteotomy, the risk of tissue overheating, thermal necrosis and soft tissue trauma remains.

Within this context, recent developments of ultrasound based osteotomy techniques have facilitated the cutting of mineralized hard tissue, while the soft tissue structures are not affected [7]. Piezoelectric bone surgery is a micrometric selective bone cutting technique using a defined ultrasonic frequency in a range of 24–32 kHz [8]. Additionally, the absence of macrovibrations, typical of rotary or oscillating instruments, make piezosurgery an advanced osteotomy technique especially suited for oral and maxillofacial applications. However, as piezo-surgical osteotomies take longer compared to conventional techniques [9], improvements have been introduced for bone cutting saw blades to provide faster, yet safe, and accurate osteotomy gaps [10].

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Hence, the purpose of our study was to compare bone healing of experimental osteotomies applying either piezosurgery or two different oscillating saw blades in a rabbit model.

The supported research hypothesis (H1) was that the use of piezosurgery and a novel acid-etched sawblade would lead to an enhanced bone remodelling in the osteotomy gap in comparison to a conventional oscillating saw. The stated null hypothesis (H0) was that early bone healing after saw osteotomy has comparable results to piezosurgery.

Material and methods

Experimental animals

The Committee on the Use of Live Animals for Teaching and Research of the University of Hong Kong (CULATR 2279-10) approved this study. Sixteen healthy adult New Zealand white rabbits, five to six months of age (4.0–4.5 kg), were used. The animals were housed in a dedicated animal holding facility under veterinary supervision in the Laboratory Animal Unit of The Li Ka Shing Faculty of Medicine, The University of Hong Kong.

Surgical instruments

For the piezo-electric osteotomies, a piezosurgery device (Piezosurgery®, Mectron Medical Technology, Carasco, Italy) was used with a power setting boosted to "burst C", i.e. maximal cutting efficiency for cortical bone, and a setting of "pump 5", which is the highest amount of water cooling during the osteotomy. All osteotomies were performed with an OT7 bone scalpel. The dimension of this insert was 0.5 mm in width and approximately 3 mm in length with five cutting teeth.

For the conventional osteotomies, an oscillating steel saw blade (532.048; Synthes®, Oberdorf, Switzerland) was used in a colibri device (Synthes®, Oberdorf, Switzerland) set. The cutting thickness was 0.4 mm. For the experimental osteotomies, a novel steel saw blade (200.054; CMT SA, Chemical Milling Technology, La Chaux-de-fonds, Switzerland) was applied. This saw blade was 0.4 mm in width (Fig. 1).

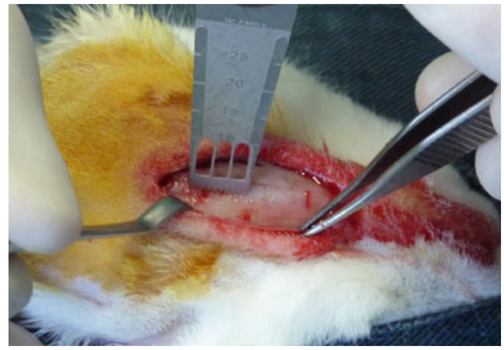

Fig. 1 Novel saw blade (200.054; CMT SA, Chemical Milling Technology, La Chaux-de-fonds, Switzerland) during osteotomy of the nasal bone. After careful preparation and elevation of skin and underlying soft tissue structures, the saw blade could be directly applied onto the bone surface. Reduced need of manual pressure and vibration allowed a safe guidance and performance of the cutting procedure

Anaesthesia

Before the surgical interventions, a subcutaneous (s/c) injection of Temgesic® (0.05 mg/kg) was performed. Intramuscular (i/m) injections of Acepromazine® (1 mg/kg) + Ketamine® (35 mg/kg) + Xylazine® (5 mg/kg) were given to anaesthetise the animals.

Post-operatively, the rabbits were observed until alert and drinking. The animals were, again, given Temgesic® (0.05 mg/kg, s/c) every 8–12 hours for the first three days post-operatively and then, followed by Metacam® injection (0.2 mg/kg, i/m), every 24 hours for a total of 14 days.

For prevention of infection, an antibiotic, Baytril® (Enrofloxacin), 5–10 mg/kg, was administered i/m every 12 hours for the first three days. Then, the antiobiotic was given in drinking water (100 mg/l) for a total of 14 days.

Surgical procedures

The 16 rabbits were randomly assigned into four groups to comply with observation periods of one, two, three and five weeks. Through a vertical midline incision of approximately 5 cm through the skin and periosteum, the naso-incisal suture line and the calvarial bone were carefully exposed.

In all animals, a total of eight osteotomies were performed (Fig. 2). Two parallel osteotomies of 20 mm and 5 mm apart from each other in the long axis of the nasal bone on one side were performed using the conventional saw blade (S1). On the other side, again, two parallel osteotomies (20 mm, 5 mm) were performed using the novel saw blade (S2). In addition, on each side of the nasal bone, the parallel osteotomy lines were connected by a perpendicular osteotomy across using piezosurgery. The same experienced surgeon performed all osteotomies (SS).

After complete transsection of the nasal bone, the periosteum and skin were repositioned and sutured in two layers.

Sacrifice

Following the observation periods of one, two, three or five weeks, the animals were killed by intravenous injection of an overdose of Pentobartital Sodium® (150 mg/kg body

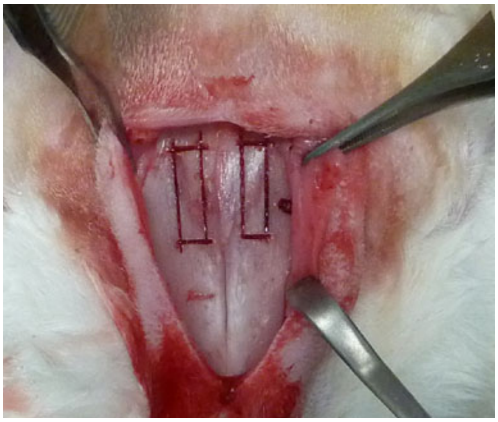

Fig. 2 Intra-operative situation after soft tissue preparation and accomplished osteotomies lines. Two pairs of parallel osteotomies of 20 mm performed by the novel (*right side*) and a conventional saw blade (*left side*). The osteotomy pairs are 5 mm apart from each other in the long axis of the nasal bone. Note the fine osteotomy line of the novel saw blade. The parallel osteotomy lines on each side were connected by a perpendicular osteotomy across applying piezosurgery. To guarantee a safe loosening and interconnection of osteotomies, the osteotomies were slightly overdesigned

weight). The nasal bone samples were harvested and fixed in 10 % para-formaldehyde solution.

Histological preparation

Subsequently, the blocks were decalcified in a solution of 14.5 % acid buffered EDTA (pH 7.2). Following this, the blocks were trimmed and cut for embedding in paraffin. The specimens were sectioned to a thickness of 6 μm, and stained with haematoxyline/eosine and toluidine blue.

Morphometric analysis

Bone healing in the osteotomy gaps was observed under light microscopy (Nikon® Eclipse LV100POL, Japan) with a digital video camera (Nikon® Digital Sight DS-Ri1, Japan) and an imaging system (NIS-Elements AR 3.00, Nikon® Laboratory Imaging software, Japan).

Each osteotomy gap was divided into three areas of equal size. Each square area of 200 × 200 μm was analysed at a magnification of 40× using a point-counting method previously described [11]. A lattice compromising 100 light points was superimposed over the tissue in each square area. The percentages of the points hitting vascularised tissue, provisional matrix (e.g. connective tissue), osteoid and mineralised bone, as well as residual tissue were calculated as previously described [12].

Statistical analysis

For the comparison of the healing outcomes of two saws with the piezo-surgical osteotomies at the four healing periods

Table 1 The percentage area occupied by various cells and tissues in osteotomy sites to identify the healing tendencies after applying piezosurgery (P), novel saw (S1) or conventional saw (S2). Amount of each group were analysed at a magnification of 40× using a point-counting method. In all three groups from one to five weeks a clear decrease of soft tissue structures and an obvious increase of new mature and mineralised tissue components could be detected

Sites	Week 1			Week 2			Week 3			Week 5		
	P	S1	S2	P	S1	S2	P	S1	S2	P	S1	S2
VT	82.3 (3.20)	0	0	0	0	0	0	0	0	0	0	0
PCT	14.3 (2.75)	65.8 (7.93)	80.8 (11.64)	72.3 (15.37)	62.3 (10.69)	59.8 (8.42)	41.8 (16.03)	46.3 (14.89)	57.5 (15.33)	41.0 (16.43)	17.8 (12.18)	22.8 (10.87)
Osteoid	0	27.5 (7.55)	17.5 (12.12)	0	0	0	0	0	0	0	0	0
MB	0	0	0	27.3 (15.11)	37.8 (10.69)	40.3 (8.42)	58.3 (16.03)	53.8 (14.97)	42.5 (15.33)	59.0 (16.43)	82.3 (12.18)	77.3 (10.87)
Residual	3.5 (1.29)	6.8 (1.26)	1.8 (0.5)	0.5 (0.58)	0	0	0	4.0 (3.65)	0	0	0	0

VT vascularised tissue with an inflammatory cell infiltrate, *PCT* provisional matrix, *osteoid* immature trabecular, *MB* mineralised bone

(one, two, three and five weeks), the percentages of various tissues within the osteotomy gaps were obtained from two (saw specimens) to three (piezo-surgery specimens) sections and averaged from the three square regions. Subsequently, mean percentages were calculated for the four animals contributing to every observation period; the animal being the unit of analysis. Two sample t-tests were used to determine the levels of significant differences (SPSS V.20).

Results

Surgery and postoperative period

In all cases, osteotomies could be performed in the predefined area without any complications. However, in comparison to piezosurgery and the novel saw blade, handling and manoeuverability of the conventional saw blade was demanding. Slight deviations and macromovements made it hard to cut the thin nasal bone in the originally planned manner. Consequently, cuts using piezosurgery and the novel saw blade looked macroscopically more precise and delicate. All rabbits recovered well.

Histological and histomorphometric findings

The histological and morphometric results at all observation periods and for the three osteotomy modalities are summarised in Table 1 and Figs. 3 and 4.

One-week specimens

After one week, in all osteotomies, bone healing was still in an early stage. The cortical matrix adjacent to the gap was very regular. There was, as yet, no mineralised bone formation within the gaps. Overall, the amount of bone debris adjacent to the osteotomy surface was very similar between the modalities. The osteotomy gap of the piezoelectric group was filled with inflammatory cells with a high degree of vascularised tissue structures (72.8 %) and provisional matrix (21.8 %). In some cases, small and scattered islands of the beginning of osteoid formation could be observed near the osteotomy rims. Osteotomy gaps of the novel saw blade (S2) revealed a high amount of provisional matrix (50.8 %). In contrast to the piezoelectric group, vascular structures were less obvious (13.6 %). However, unimpaired bone vitality was demonstrated by newly formed osteoid (26.9 %), which was regularly found adjacent to the osteotomy surface. Results of the conventional saw blade (S1) revealed similar findings. Provisional matrix dominated (50.9 %) in the gap. Vascularised structures were more pronounced (22.3 %) and osteoid formation (22.3 %) started from the periosteal aspect. Additionally, in some cases, some small edges and fractured bone debris was detectable near the surfaces, most likely caused by the strong vibrations of the saw.

Two-weeks specimens

In all three groups a considerable amount of newly formed osteoid and slightly mineralised bone formation could be detected without complete bridging of the

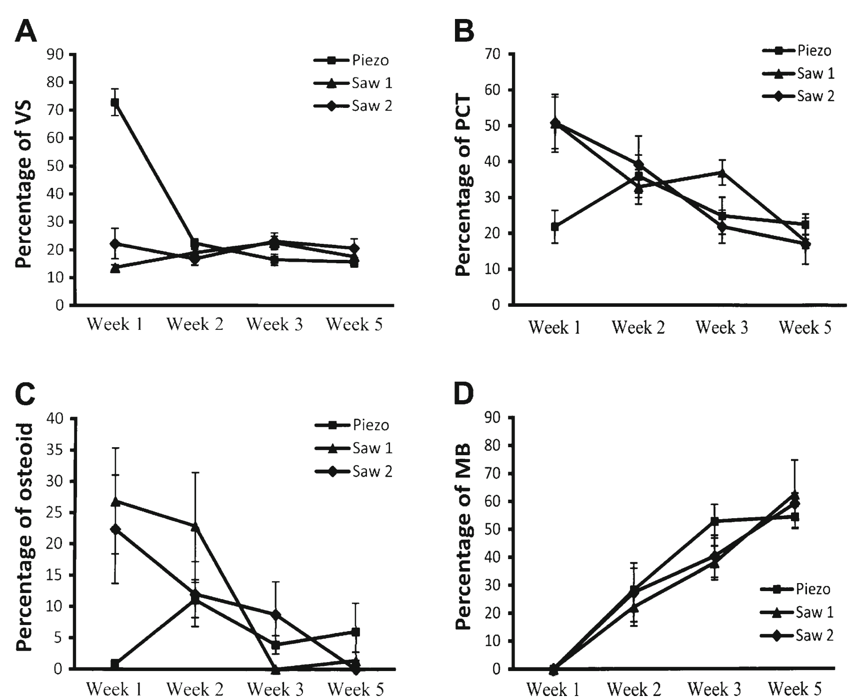

Fig. 3 Percentages of analysed tissue components within the osteotomy gaps during the observation period from one to five weeks. In each table the development and amount of tissue structures are summarised with standard deviations (*whiskers*) after applying conventional saw, novel saw, or piezosurgery. Individual values of each technique are summarised in Table 1. **a** Vascular structures (VS). **b** Provisional matrix (PCT). **c** Osteoid. **d** Mineralised bone (MB). Piezosurgery; Saw 1 = novel saw blade (CMT); Saw 2 = conventional saw blade (Synthes®)

osteotomy gap. The mineralised areas were regularly found adjacent to the osteotomy surfaces. In all groups the amount of bone debris was reduced by ongoing osteoclastic activity. The piezoelectric group revealed a high bone remodelling activity. Whereas the initial amount of vascular structures decreased (22.4 %), new osteoid (11.1 %) and mineralised bone tissue (28.3 %) had increased. The gap was mostly filled with a mixture of provisional matrix (36.0 %) and immature woven bony structures.

In contrast, in the S2 group, vascular structures increased (19.0 %) and osteoid (22.9 %) decreased from one to two weeks. Also, the amount of provisional matrix declined (33.0 %). The osteotomy gap was filled with a dense composition of soft tissue structures with distinct areas of early mineralisation (22.1 %). A tightly arranged seam of osteoid and loose woven bone on the osteotomy rims was visible.

In the S1 group, the amount of vascular (16.8 %) and provisional matrix (39.3 %) tissue structures diminished

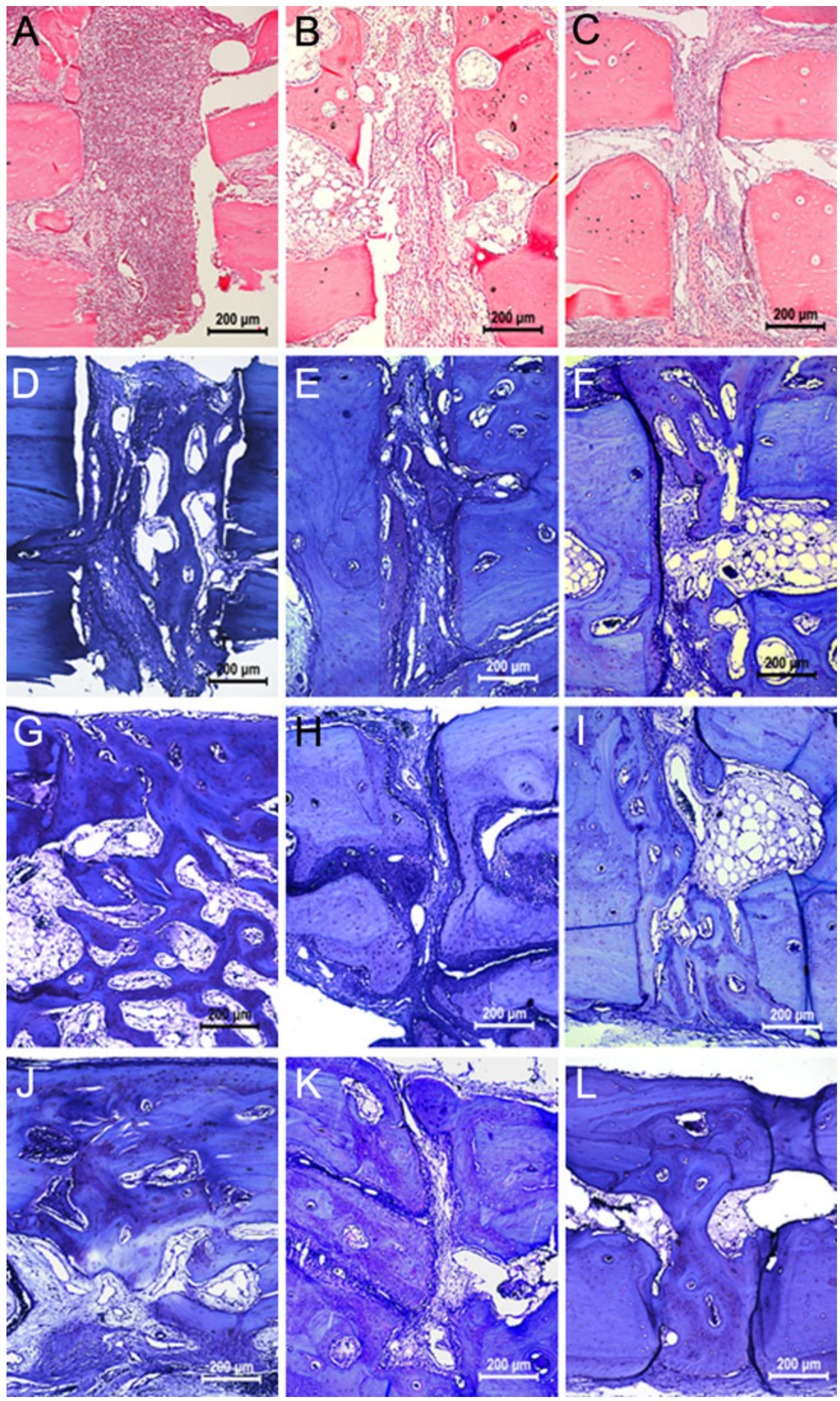

Fig. 4 Summary of histological results. Specimens after 1 week (**a**, **b**, **c**) were stained with haematoxyline-eosine to clearly identify soft tissue structures and matrix in the early healing phase. Specimens after 2 weeks (**d**, **e**, **f**), 3 weeks (**g**, **h**, **i**) and 5 weeks (**j**, **k**, **l**) were stained with Toluidine blue to evaluate new bone formation and bone remodelling. The left column represents piezosurgery (**a**, **d**, **g**, **j**), the middle column the novel saw blade (**b**, **e**, **h**, **k**) and the right column the conventional saw blade (**c**, **f**, **i**, **l**). All three osteotomy techniques disclosed a characteristic gap healing with bridging of the osteotomy site with immature and woven bone in the initial healing stage. After five weeks all techniques revealed an advanced remodelling with lamellar structures

from one to two weeks. Also the mass of osteoid (12 %) showed a clear reduction. Distinct woven bone formation (27.4 %) was spreading from the periosteal aspects and was closely attached to the osteotomy surface.

Three-weeks specimens

At week three, osteotomy gaps were bridged by bony tissue structures in all three groups. An advanced stage of primary gap healing with newly formed woven bone surrounded with less fibro-vascular tissues and minimal bone debris was visible. In the piezoelectric group, the osteotomy gap was almost completely filled with bony structures. More than half (52.8 %) of the newly formed tissue disclosed intermediate mineralisation. Only less soft tissue structures (vascular, 16.5 %; provisional matrix, 24.9 %) accounted for the overall presentation of the osteotomy gap. In some specimens, consolidation of the osteotomy site was in an advanced stage so that the osteotomy lines could hardly be recognised.

Bone healing in the S2 group disclosed a very similar healing pattern with a progressive process of mineralisation (37.9 %). However, in contrast to the piezoelectric group, the osteotomy gap was still visible with a clear streak of connective tissue mainly composed of provisional matrix (37 %). Quantitatively, no osteoid could be detected (0 %).

In the S1 group, connective tissue structures were less pronounced (provisional matrix, 21.9 %). Even though gap healing was advanced, the osteotomy gap was still definable. In particular, the rims of the osteotomy gap showed intense remodelling with plenty of new mineralised bone (40.3 %).

Five-weeks specimens

At week five, osteotomy gaps were almost completely filled with newly formed bone in all three groups. The new bone exhibited a high degree of mineralisation with some lamellar structures, especially in the piezoelectric group (Fig. 5). No remnants of initial bone debris were present. In comparison to three weeks, bone mineralisation in the piezoelectric group accounted for 54.3 %. Consolidation of the osteotomy was in many cases almost complete.

Findings in both saw groups were similar. Mineralised bone formation/maturation could be seen within the gaps. In both groups, the amount of connective tissue structures was further depleted, whereas the degree of mineralisation rose. Woven bone structures already disclosed early lamellar orientation (remodelling).

Discussion

It was the aim of this experimental study to compare bone healing of experimental osteotomies using either piezosurgery or two different oscillating saw blades in a rabbit model. All three osteotomy techniques revealed an advanced gap healing already starting after one week. The most pronounced new bone formation took place between two and three weeks, whereby piezoelectric surgery revealed a tendency to faster remodelling. Yet, there were no statistically significant differences between the groups.

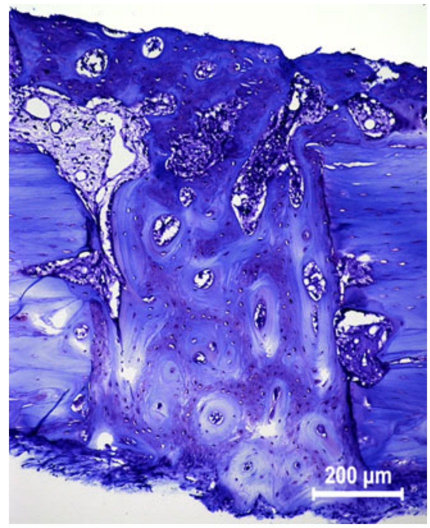

Fig. 5 Five-week gap healing after piezosurgery. The osteotomy gap is almost completely filled with mature mineralised bone undergoing remodelling. Toluidine blue, 40×

To study fracture healing several animal models are described in literature [13, 14]. For the analysis of bone remodelling after applying different bone cutting instruments, commonly a tibial osteotomy is performed either in sheep or rabbits [15, 16]. Whereas sheep have the advantage that they exhibit a similar lamellar bone structure and bone metabolism rate to humans [17], rabbits represent an economical valuable model offering adequate quantities and qualities of tissue for initial pretranslational research [18]. In our study, instead of a tibial osteotomy a nasal bone osteotomy in rabbits was performed to evaluate the manoeuverability and precision of the saw blades for cutting thin and fragile bone structures. Usually, this surgical approach is chosen for access to the sinus cavity for a later augmentation procedure [19]. The bones of the cranial vault offer the opportunity to analyse the healing characteristics of intramembranous instead of enchondral bone remodelling [20]. As healing of long bones and flat bones reveals some differences, the selected model allowed a reasonable comparison with other studies evaluating bone cutting in the cranio-facial region.

The findings of our study supported and proved an undisturbed and fast bone healing of the piezoelectric osteotomy. There were no signs of thermal damage at all. Furthermore, the handling and precision of the piezoelectric osteotomy was in accordance with results of other authors [21].

In terms of handling and performance of the two saw blades, the new saw blade revealed less initial trauma. As this is the first *in vivo* report analysing the efficiency of this novel saw blade, no further comparisons could be made at this time. However, as there is an urgent need for less traumatic and minimally invasive bone cutting techniques, especially for corrective or reconstructive purposes for developmental deformities, the characteristic performance of the novel saw blade might render it as a suitable tool for a gentle osteotomy.

Moreover, in the course of recent developments of image-guided, computer-navigated and robotic-assisted bone surgery, the need for highly precise, safe and accurate cutting instruments is urgently required [22, 23]. Only if the applied devices allow a fast and safe osteotomy with limited damage to surrounding tissues, may they be used for such indications. Owing to their great manoeuverability in the recent past, lasers and high-pressure water jets have been evaluated. However, even though results especially for certain laser systems were promising [24], in daily routine oscillating and ultrasound based systems are still preferred by surgeons. Osteotomy speed and depth control using saw blades is still unrivalled. Therefore, if particularly accuracy and soft tissue preservation with unchanged cutting performance of saw blades could be achieved, this would finally render new opportunities for a fast and safe osteotomy of delicate bone structures in orthopaedics, oral and maxillofacial surgical arena.

Conclusion

In our study the use of a novel as well as the piezoelectric bone cutting instrument revealed advanced bone healing with a favourable surgical performance for safely cutting thin and fragile bone structures in a small animal model.

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