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Research Article

Assessment of Five Different Ultrasonic Bone Osteotomies in Rabbit Skull - Micromorphological Evaluation

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Abstract

Purpose: The novel ultrasonic osteotomy technique called piezosurgery is an alternative to conventional osteotomy devices. The aim of this study was to compare the micromorphology after the use of five different ultrasonic osteotomy in rabbit skulls.

Materials and Methods: Fresh bone samples were taken from a rabbit skull using the Piezosurgery® 3 (insert tip - OT7), Piezosurgery® Medical (insert tip - MT1-10), Piezon Master Surgery® (insert tip - SL1), VarioSurg® (insert tip - SG1), and Piezotome® 2 (insert tip - BS1 II). For conventional histological analysis Masson-Goldner Trichrome staining was performed. Additionally, the bone surfaces were examined using a dark field microscope.

Results: The histological analysis of the stained bone samples as well as the dark field microscopic examinations of the unmodified bone samples revealed typical calvarial bone structure with compact (external and internal) and spongy (diploe) bones. Minor differences between the tested ultrasonic devices could be observed regarding the amount of bone debris and the integrity of cancellous bone within the osteotomy line.

Conclusion: In the present study, minor micromorphological differences following the use of five ultrasonic devices could be identified and due to the bone micro-architecture preservation found all tested devices might facilitate bone healing.

Keywords: Bone Micromorphology; Ultrasonic Osteotomy; Rabbit Skull; Dark Field Microscopy; Piezosurgery

Introduction

The transplantation of autogenous bone harvested from various intra- and extraoral graft regions is considered to be the gold standard for reconstructive procedures of the alveolar ridge [1]. This transplanting requires osteotomies at the donor and recipient sites. Like any other osteotomy, these procedures are associated with the risk of harming vulnerable structures, such as nerves, blood vessels and sinus membrane. Using conventional osteotomy techniques, iatrogenic lesions of such vulnerable structures cannot always be avoided [2]. Due to its selective cutting effect of mineralized tissue, ultrasonic osteotomies may be an alternative to such conventional osteotomies. This ultrasonic bone cutting was introduced more than 30 years ago by Horton., *et al.* [3] and applied for practical use by Vercellotti., *et al* [4].

Ultrasonic osteotomy is based on the reciprocal piezo effect. Deformation of a piezoelectric crystal in an electric field creates an alternate and perpendicular expansion and contraction of the material.

The number of available ultrasonic devices in the world market has recently increased bringing improved instruments impacting how osteotomies are performed worldwide. *Ex vivo* studies have revealed differences between conventional osteotomes, such as rotating or sawing devices, and piezoelectric osteotomes (Piezosurgery®) regarding the micromorphology and roughness values of osteotomized bone surfaces [5] and bone structure integrity may considerably affect bone healing [3].

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Hence, this present study compares the micromorphologies of osteotomized bone surfaces after the application of five different ultrasonic osteotomies by back light microscopy.

Materials and Methods

Fifteen healthy, half-year-old, female White New Zealand Rabbits with an average body weight of 5.13 ± 0.21 kg (mean ± standard deviation) were used. This study was approved by the pertinent authorities (Registration No.: 222-2684-04-014-004/06, Thüringer Landesamt für Lebensmittelsicherheit und Verbraucherschutz, Germany). Immediately after intravenous euthanasia injection of 5 ml of T61® (Hoechst AG; Frankfurt, Germany), two bone samples of each rabbit skull, measuring 6 x 6 mm, were taken via osteotomies under sterile 0,9% saline solution irrigation and careful detachment from the meninges (Figure 1). Six bone samples (three animals) were prepared using one of these five different ultrasonic devices: Piezosurgery® 3 (Mectron Medical Technology, Carasco, Italy) with assembled insert tip OT7, Piezosurgery® Medical (Mectron Medical Technology, Carasco, Italy) with assembled insert MT1-10, Piezon Master Surgery® (EMS, Nyon, Switzerland) with assembled insert tip SL1, VarioSurg® (NKS, Tochigi, Japan) with assembled inert tip SG1, and Piezotome® 2 (Acteon Group, Bordeaux, France) with assembled insert tip BS1 II. All five insert tips are depicted in figure 2. The total of bone samples prepared was thirty (n = 30). Table 1 summarizes the characteristics of the ultrasonic osteotomes in terms of frequency, power, number of piezo ceramics, in addition to the thickness and number of teeth of each insert tip. All osteotomies were performed by only one single surgeon using the ultrasonic devices in the boosted mode at a maximum vibration frequency and irrigation (0.9% sterile saline solution) as provided by each device.

	1	2	3	4	5
Device name	Piezosurgery® 3	Piezosurgery® Medical	Piezon Master Surgery®	Variosurg®	Piezotome® 2
Manufacturer	Mectron	Mectron	EMS	NSK	Acteon
Frequency	24-36 kHz	24-36 kHz	24-32 kHz	27-34,5 kHz	28-36 kHz
Power	25 W	25 W	25 W	17 W	60 W
Number of piezo-ceramics	4	4	4	4	6
Insert tip	OT7	MT1-10	SL1	SG1	BS1 II
Thickness	0.5 mm	0.5 mm	NA	0.5 mm	0.7 mm
Teeth	5	5	5	5	4
Amplitudes	40 µm	40 µm	NA	90 µm	30-60 µm

Table 1: *Characteristics of the investigated ultrasonic devices.*

NA = Not available.

Figure 1: *Intraoperative view of a rabbit skull with marked bony specimens before ultrasonic osteotomy.*

Figure 2: *Five different ultrasonic devices with their respective insert tips (from left to right): the Piezosurgery® Medical (MT 1-10), Piezosurgery® 3 (OT7), Piezotome® (BS-1-II), VarioSurg® (SG-1), and Piezon Master Surgery® (SL-1).*

The native bone samples were stored at 4°C. of each bone square. Two 1.0 mm-thick bone strips were prepared coplanar to the osteotomized surface using a conventional diamond cutting disc under ample cooling via a 0.9% sterile saline solution, which were subsequently fixed to the osteotomized surface parallel to the slide and stereo optically examined via dark field microscopy (Leica DMRD/DMRXE, Leica Microsystems Wetzlar GmbH, Wetzlar, Germany). Analysis was performed with the software package analySIS Pro 3.2 (Olympus Soft Imaging Software GmbH, Hamburg, Germany).

One mm-thick bone strip of each group was examined by a contact profilometer (AMBIOS Technology, Santa Cruz, CA - USA). Contact profilometry was particularly chosen because of its capability of non-destructive surface characterization. To determine the primary roughness parameters and the waviness, a contact profilometer was used under the terms of DIN EN ISO 13561-1. To avoid any surface damage, the stylus tracking force was limited to 25 mN with a stylus diameter of 2.5 µm. One line profile of the cortical bone area of each bone sample was recorded. A length of 1 mm was chosen because of its reliability, accordingly with the preliminary tests. The root mean square Rq was calculated from the acquired primary profile data (DIN EN ISO 4287:2010-07) as expressed by the equation below:

$$Rx = \sqrt{\tfrac{1}{2} \int_0^1 | Z^2(x) | dx}$$

The number one in the formula means the total length measured and a Z(x) the discrete height value at position x. For each of the different bone strips 18900 single value Z(x) was detected over the measured distance. The choice of root-mean-squared roughness as the describing parameter allows by simply adding the individual measured distances of each sample type as well as the corresponding Z(x)-values to specifying a common statistical value for each type of cutting technique/device.

Bone samples were fixed with 4% (v/v) paraformaldehyde for two weeks for light microscopy. After decalcification with 15% (m/v) ethylenediaminetetraacetate (EDTA) for fourteen days and washing with 0.1M phosphate buffered saline (pH = 7.4), all samples were dehydrated with ascending aqueous ethanol (50%-70%-80%-90%-96%-100%) and xylene for a total of four days. Afterwards, the bone samples were embedded in paraffin and cooled down at room temperature. Each surface of interest was identified, and two 5 µm thin slices of each sample were cut using a slide microtome. Masson-Goldner Trichrome staining was used to enable evaluation of bone structures. The histological analyses were performed using a light microscope (BH-2, Olympus, Hamburg, Germany) and imaging software (cell^A 2.8, Olympus Soft Imaging Solution GmbH, Hamburg, Germany). Images were made at 400X magnifications.

Results

Masson-Goldner Trichrome staining revealed the preservation of delicate cancellous diploe bone structure in each of all surfaces of the osteotomy sites created by the five investigated ultrasonic devices (Figure 3a-3e). After using Piezosurgery® 3 and Piezon Master Surgery® a smooth osteotomized bone surface with intact trabecular architecture could be detected (Figures 3a and 3b). The VarioSurg®, Piezotome® 2 and Piezosurgery® Medical devices caused few microfractures limited to the osteotomy line within the cancellous bone layer (Figures 3c-3e).

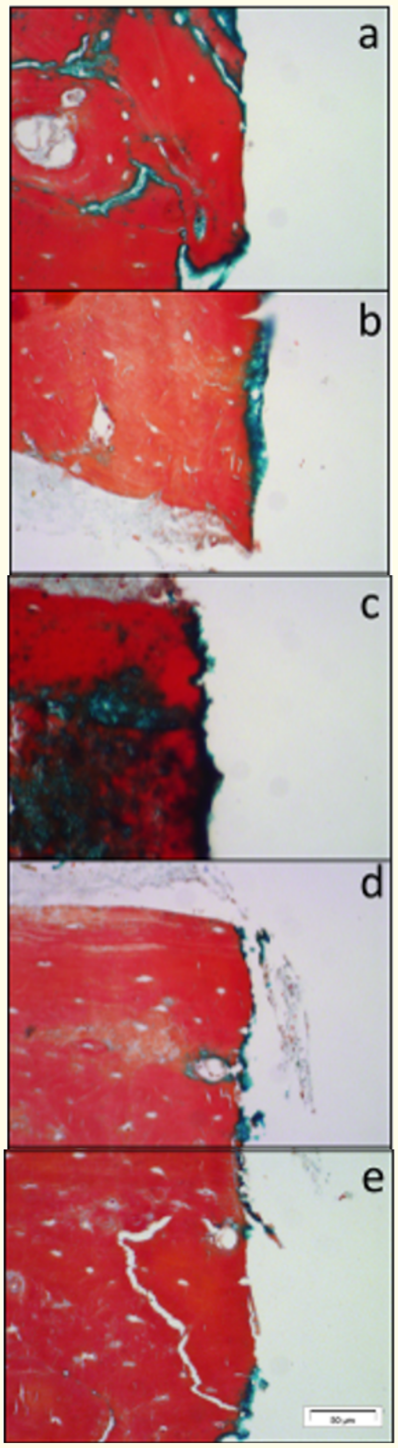

Figure 3: *Light microscopy of the bone surface after ultrasonic osteotomy (decalcified section, Masson-Goldner Trichrome stainning, scale = 50 µm): a) Piezosurgery® 3; b) Piezon Master Surgery®; c) VarioSurg®; d) Piezotome® 2; e) Piezosurgery® Medical.*

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Dark field microscopy of the osteotomized bone samples revealed a typical calvaria bone structure, including tabula externa, diploe, and tabula interna in all cases (Figures 4a-4e). However, although the osteotomized bone surfaces showed nearly identical micromorphological features, minor differences could be identified. After using Piezosurgery® 3 and Piezon Master Surgery®, cancellous bone was nearly free of bone debris (Figure 4a and 4b). After osteotomy via Piezosurgery® Medical device some bone debris could be detected (Figure 4e). When Piezotome® 2 and VarioSurg® were used (Figures 4c and 4d) the amount of bone debris were slightly increased and the osteotomized surfaces appeared rougher than those obtained with other devices.

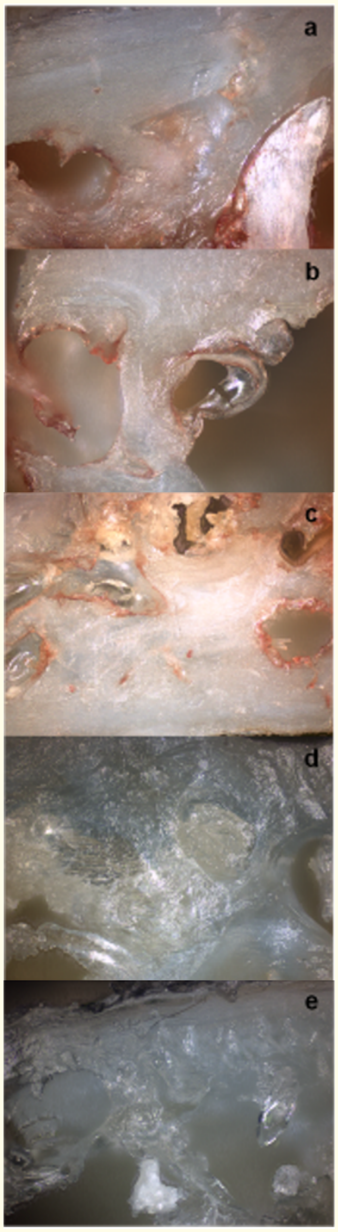

Figure 4: *Dark field microscopy of the bone surface after ultrasonic osteotomy (unmodified bone samples, scale = 200 µm): a) Piezosurgery® 3; b) Piezon Master Surgery®; c) VarioSurg®; d) Piezotome® 2; e) Piezosurgery® Medical.*

Discussion

The suitability of all five investigated ultrasonic osteotomies have been confirmed by several different clinical studies [2,6]. Ultrasonic osteotomy devices use a modulated ultrasonic frequency that permits highly precise and safe cutting of mineralized tissue [6]. Due to the prevention of surrounding soft tissue ultrasonic osteotomy is especially suitable for interventions in the vicinity to vulnerable structures such as nerves, blood vessels, meninges and sinus membrane [7,8]. In the present study, all tested ultrasonic devices created a very fine osteotomy line. The integrity of the meninges was preserved in all cases confirming the ability of selective cutting of mineralized tissue.

Moreover, earlier published studies revealed micromorphological differences of the osteotomy sites comparing conventional and ultrasonic osteotomies. These differences might have an impact on bone healing and reossification [5]. In the present study, five different ultrasonic osteotomes were evaluated with regard to the micromorphology of the osteotomized surfaces. The rabbit skull was chosen to simulate the clinical situation of calvarial bone grafting [9]. To evaluate the bone microstructure, dark field microscopy was performed. Similar to the established reflected-light microscopy, dark field microscopy allows the three-dimensional reconstruction of specimens. However, reflected-light microscopy uses one orthogonal light beam. Hence, light reflected by particles in the same z-axis but different depths interferes resulting in a summations effect. In contrast, dark field microscopy, which is widely used in material science, is based on a cone of light that focuses on the sample. Therefore, the summations effect of light reflected by particles in the same Z-axis but in different depths is avoided resulting in a clearer contrast and hence an even better three-dimensional imaging compared to the reflected-light microscopy.

Former histological examinations of conventional osteotomies at the rabbit skull revealed totally condensed osteotomized surfaces. Hence, the calvarial bone structure with its compact and cancellous bone layers were hardly identified. The cancellous spaces were filled with a large amount of bone debris. Parallel impressions caused by a saw were observed over the entire surface. The use of a rotating instrument resulted in partially destroyed trabecular structures of the cancellous bone. The typical bone micro-architecture was barely identified [10]. In contrast, all five investigated devices preserved the osseous micro-structure. The compact and cancellous bone layers could be easily differentiated. Within the cancellous bone layer, the trabecular structures were almost completely preserved and the amount of bone debris was clearly decreased compared to the previously published conventionally osteotomized bone samples. Nevertheless, few microfractures and slightly more bone debris were found after the use of Variosurg®, Piezotome® 2, and Piezosurgery® Medical.

The observed reduction of bone debris in comparison to conventional osteotomies expressed in other articles could positively impact the centrifugal blood supply of the bone [11]. Blood irrigation and cell migration might be facilitated by preserving the integrity of the cancellous bone, which can positively impact the bone healing processes due to the high osteogenic potency of cancellous bone [12]. The observed preservation of the bone microstructure may less significantly impair the complex signaling cascade of cytokines and growth factors that start each bone healing process [13,14]. Although proinflammatory cytokines are necessary in early bone healing, an increased and persistent expression of proinflammatory cytokines and chemokines that are caused by various pathogenic factors compromises bone healing. Thus, a decreased inflammatory reaction following a less traumatic osteotomy may result in a more prompt and profound bone healing [15]. Less severe inflammatory response may facilitate osseointegration and reduce the risk for a peri-implantitis [16], which is a major risk factor for implant loss. The observed minor micromorphological differences between the tested ultrasonic devices may have an influence on bone healing.

The specific reasons why Piezosurgery® 3 and Piezon Master Surgery® produced none or almost no bone debris after used for rabbit skull osteotomies were none to be found and could be related directly to the characteristics of those two devices.

Further *in vivo* studies with larger number of samples regarding to bone healing after ultrasonic osteotomies are required.

Conclusion

All five tested devices are suitable for fine osteotomy lines and selective cutting of mineralized bone tissue with Piezosurgery® 3 and Piezon Master Surgery® producing none or almost no debris after its use. Due to the demonstrated preservation of the bone micro-architecture, all tested devices might facilitate bone healing.

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