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Title: ASSESSMENT OF NERVE DAMAGE USING A NOVEL ULTRASONIC DEVICE FOR BONE CUTTING

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Corresponding Author: Dr. Stefan Schaeren, MD

Corresponding Author's Institution: University Hospital

First Author: Stefan Schaeren, MD

Order of Authors: Stefan Schaeren, MD; Claude Jaquiéry, M.D., D.D.S.; Michael Heberer, M.D.; Markus Tolnay, M.D.; Tomaso Vercellotti, D.D.S.; Ivan Martin, PhD

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Abstract: Objectives. To assess the potential damage of Piezosurgery®, a novel piezoelectric instrument for bone cutting, to a peripheral nerve upon direct contact.

Material and Methods. The sciatic nerves of male Wistar rats were exposed to Piezosurgery® at the typical force applied during osteotomies, simulating accidental contact to the nerve without immediate retraction, or at a peak force, simulating a hit on the nerve due to accidental slip on bone tissue. Contact with the nerve was also applied without ultrasonic activation. Motor and sensory nerve damage were assessed respectively by walking track analysis with quantification of the Sciatic Function Index (SFI) and by withdrawal reflex tests, up to 150 days after injury or until normalized function. Animals (8 per group) were then sacrificed and the exposed nerves analysed histologically. Differences in SFI and histological scoring were statistically assessed by Mann-Whitney tests.

Results. Direct exposure of peripheral nerve to Piezosurgery® did not dissect the nerve, but induced structural and functional damage. The frequency and extent of functional damage were higher with increased pressure applied on the nerve, but not by activation of ultrasonic vibration. Various degrees of axonal damage were observed in all groups, without a consistent relationship with functional deficits. Importantly, since the perineurium was always intact, functional recovery was almost complete within 60 days in all groups.

Conclusion. Our results indicate that accidental contact of Piezosurgery® with the nerve is not necessarily detrimental, and the potential for functional recovery is large. Thus, the safety margins would be larger than using instruments which are typically operated at higher force (e.g., bur) or which are likely to cut the nerve upon direct contact (e.g., oscillating saw).

* Revision Cover Letter (with Revision Notes)

Universitätsspital Basel

Behandlungszentrum Bewegungsapparat

Wirbelsäule

Spitalstr. 21
CH-4031 Basel
Telefon ++41 61 265 78 10
Telefax ++41 61 265 78 09

Dr. Stefan Schären
E-Mail sschaeren@uhbs.ch

Dr Leon A. Assael
Oral and Maxillofacial Surgery
School of Dentistry—SD-522
Oregon Health & Science University
611 SW Campus Dr
Portland, OR 97239

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Dear Dr. Assael,

Thank you for the favorable review of our manuscript. The constructive comments of the Reviewer have been taken into account for the preparation of a revised version. In particular, we have (i) added Figure 2, with histological sections depicting the different degree of neural damage and (ii) included 2 additional references (# 4 and 5).
We are looking forward to hearing from you.

Yours sincerely,

Dr. Stefan Schaeren

ASSESSMENT OF NERVE DAMAGE USING A NOVEL ULTRASONIC DEVICE FOR BONE CUTTING

[*]Stefan Schaeren, MD, [1 *]Claude Jaquiéry, MD, DDS, [1] Michael Heberer, MD, [1] Markus Tolnay, MD, [2]Tomaso Vercellotti, DDS, [3]Ivan Martin, PhD[1]

[1] Departments of Surgery and of Research, University Hospital Basel, Switzerland

[2] Institute of Pathology, University Hospital Basel, Switzerland

[3] Private Practice, Genova, Italy

[*] = Equally contributing authors

Corresponding author:

Stefan Schaeren, MD

Clinic of Orthopaedic Surgery

University Hospital Basel

Spitalstrasse 21

CH-4031 Basel - Switzerland

Mail: sschaeren@uhbs.ch

Phone: +41(0)61 2652525

Fax: +41(0)61 2657809

Key Words: ultrasonic surgery, nerve injury, sciatic nerve, functional recovery, walking track analysis

Abstract

Objectives. To assess the potential damage of Piezosurgery®, a novel piezoelectric instrument for bone cutting, to a peripheral nerve upon direct contact.

Material and Methods. The sciatic nerves of male Wistar rats were exposed to Piezosurgery® at the typical force applied during osteotomies, simulating accidental contact to the nerve without immediate retraction, or at a peak force, simulating a hit on the nerve due to accidental slip on bone tissue. Contact with the nerve was also applied without ultrasonic activation. Motor and sensory nerve damage were assessed respectively by walking track analysis with quantification of the Sciatic Function Index (SFI) and by withdrawal reflex tests, up to 150 days after injury or until normalized function. Animals (8 per group) were then sacrificed and the exposed nerves analysed histologically. Differences in SFI and histological scoring were statistically assessed by Mann-Whitney tests.

Results. Direct exposure of peripheral nerve to Piezosurgery® did not dissect the nerve, but induced structural and functional damage. The frequency and extent of functional damage were higher with increased pressure applied on the nerve, but not by activation of ultrasonic vibration. Various degrees of axonal damage were observed in all groups, without a consistent relationship with functional deficits. Importantly, since the perineurium was always intact, functional recovery was almost complete within 60 days in all groups.

Conclusion. Our results indicate that accidental contact of Piezosurgery® with the nerve is not necessarily detrimental, and the potential for functional recovery is large. Thus, the safety margins would be larger than using instruments which are typically operated at higher force (e.g., bur) or which are likely to cut the nerve upon direct contact (e.g., oscillating saw).

Introduction

In oral, maxillofacial and spinal surgery many osteotomies have to be performed in close vicinity to nerve tissue, with the potential risk for transient or permanent neurological injuries, e.g. to trigeminal nerve branches.[1] Traditional tools such as rotating burs or oscillating saws are highly effective in cutting bone tissue but are not selective for bone and thus can produce important harm to surrounding soft tissues, especially nerves.

Piezosurgery®, a device for bone-cutting based on low-frequency (25-29 kHz) ultrasonic vibrations, has recently been introduced in oral and maxillofacial surgery. The method enhances control and precision of osteotomies,[2] as well as bone healing, due to reduced local trauma.[3,4] In a recent in vitro study, the use of Piezosurgery® for transposition of the inferior alveolar nerve in the mandibles of cadaver sheep resulted in roughening of the epineurium without affecting deeper structures, and induced a lower degree of injury than when using a conventional rotary bur.[5] Furthermore, in a pilot clinical study, the use of Piezosurgery® resulted in reduced neurosensory disturbances in orthognathic surgery of the mandible.[6] However, to the best of our knowledge no experimental in vivo study has yet demonstrated that bone cutting using Piezosurgery® prevents damage to soft tissue, especially to nerves.

Aim of this study was to assess the potential damage of Piezosurgery® to a peripheral nerve upon direct contact, in two possible scenarios. The first scenario corresponds to the condition when the surgeon does not immediately realize of the contact with the nerve and continues to apply the same force used for cutting bone (*working force*) for a reaction time estimated to be of 5 seconds. The second scenario corresponds to an accidental slip of the device on the bone tissue, causing a direct hit on the nerve with a higher force (*peak force*), estimated to be 2-fold of the working force, but for a shorter time (1 second). The sciatic nerve of the rat was selected as an established model for studying sensory and motor nerve damage and recovery,[7] also considering that the size of the sciatic nerve of the rat is equivalent to the size of branches of human trigeminal nerve.

Materials and Methods

Operation of the ultrasonic instrument

Piezosurgery® (Mebiotec Srl, Sestri Levante, Italy) was developed to perform osteotomies by application of micrometric ultrasonic piezoelectric vibrations.[2] In our study, the tool was used at the highest possible frequency (i.e., 29 kHz) and cutting energy (i.e., boosted mode), in order to simulate the worst-case scenario, and with the cooling irrigation system set at 60 mL/min of sterile solution flow, to prevent heat damage.

In order to determine the average force applied to perform osteotomies, the piezoelectric device was mounted on a custom-made holder, with a load cell (Honeywell Inc., Il, USA) fixed to the handle. The load signal during operation was digitally converted and recorded using PICO Software (Pico Technology Limited, St. Neots, UK). The typical *working force*, determined as the average force applied by 2 trained surgeons performing 5 osteotomies on a calvarial bone of a rabbit, was 1.5N ± 0.3N. The *peak force*, simulating slipping on the bone and hitting of the nerve, was assumed to be double of the working force (i.e., 3.0 N).

Animal treatment

A total of 25 male Wistar rats (average weight 360 g) were operated on the left hind foot under isoflurane anaesthesia (1.5-3.5 Vol% Isofluran with 0.6 l/min O_2, delivered by means of a mask), following protocol approval by the local ethical committee. The surgical site was shaved and disinfected with 10% povidone-iodine solution (Betadine, Mundipharma). Following skin incision, the sciatic nerve was exposed by blunt dissection for a length of 2.5 cm proximal to its division into the tibial and the peroneal nerve, and dissected from the underlying soft tissues. In order to prevent slipping of the nerve during exposure to Piezosurgery®, a wooden spatula (0.5 cm width x 5cm length x 0.15cm height) was placed under the nerve, acting as a buttress. The nerve was then exposed to the piezoelectric instrument, according to the following experimental groups:

Group **A:** contact only with Piezosurgery® non active, *working force* for 5 sec (N=8 animals).

Group **B:** contact and ultrasonic activation of Piezosurgery®, *working force* for 5 sec (N=9 animals).

Group **C:** contact and ultrasonic activation of Piezosurgery®, *peak force* for 1 sec (N=8 animals).

It should be pointed out that the duration of contact in groups A and B corresponds to a worst-case scenario, since a skilled surgeon typically retracts the instrument in a shorter time. Time of exposure and applied force were measured and found to be within 10% of the nominal values. The surgical site was then rinsed with sodium chloride and the wound closed in two layers using Prolen 3.0 sutures (Ethicon). For pain relief, animals received Buprenorphin 0.1 mg/kg s.c. after closing the wound and 12 and 24 hours postoperatively. After the operation, animals were kept in separate cages, with free access to food and water.

Functional tests

Motor and sensory nerve function were monitored on the first postoperative day and at timed intervals, up to 150 days after injury or until repeated normalized function. Motor nerve function was quantified by calculation of the Sciatic Function Index (SFI) based on walking track analysis, as previously described.[8] Values lower than -10 were considered to indicate a motor damage. Sensory nerve function was assessed by withdrawal reflex tests. Tests were performed three times per animal by applying a current of up to 1 mA, at incremental steps of 0.1mA/sec, first to the untreated foot and then to the operated one. A reproducible difference in the threshold of sensitivity greater than 0.1mA between the two feet was considered to indicate a sensory damage.[9]

Histological assessment

When motor and sensory function was fully recovered or 150 days after surgery, animals were sacrificed in a CO_2 euthanasia system. The exposed sciatic nerves were harvested, fixed in 4% formalin, embedded in paraffin, longitudinally sectioned (5 um thick) and stained for hematoxylin-eosin and Holmes-Luxol (stains myelin and axons). Sections were analyzed by a neuropathologist (MT) for the integrity of the perineurium and axonal damage. The axonal damage was graded into 0= no damage, 1= minor axonal damage (axonal breakdown, "digestion chambers", fiber loss), 2= severe axonal damage but not covering the whole nerve fascicles , 3= severe axonal damage covering the whole nerve fascicles.

Statistics

Differences in SFI were statistically assessed by Mann-Whitney tests, and considered significant with $p<0.05$.

Results and Discussion

The postoperative phase was uneventful in all cases presented. Only one animal in group **B** with clear motor and sensory deficit mutilated itself three days postoperatively, and was thus sacrificed and replaced.

Motor and sensory nerve functions of the treated animals are displayed in Fig. 1 and can be summarized as follows. Group **A** (*working force* for 5 sec, Piezosurgery® not active)**:** postoperatively, 4 out of 8 animals showed motor and/or sensory deficits. These 4 animals recovered within 20 days. Group **B** (*working force* for 5 sec, Piezosurgery® active): postoperatively, 5 out of 8 animals showed motor and/or sensory deficits. Four of these animals fully recovered within 60 days, and only 1 remained pathological with respect to motor function. Group **C** (*peak force* for 1 sec, Piezosurgery® active)**:** postoperatively, all animals had motor and/or sensory deficits. Seven animals fully recovered within 60 days, and only 1 remained pathological with respect to motor function. No further recovery of motor or sensory damage was found in any group between 60 days and 150 days. Animals in group **C** displayed a higher occurrence of functional damage than those in groups **A** or **B** up to 30 days after the treatment. Moreover, at 10 days the Sciatic Function Index (SFI) of the pathological animals was significantly lower in group **C** than in groups **A** and **B**, indicating an increased extent of motor function damage by nerve exposure to a *peak force*. Instead, both frequency of functional damage and SFI were similar in groups **A** and **B**, indicating a limited effect induced by activation of the ultrasonic vibration.

Histologically, no dissection or damage of the perineurium was visible in any of the nerves of groups **A**, **B** or **C**. Degeneration of nerve fibers in restricted areas was observed in all animals and ranged from isolated (Fig. 2A) to abundant axonal breakdown and segmentation of myelin into digestion chambers (Fig. 2B). However, no systematic difference was found among the groups or in relation to a functional deficit. Semiquantitative scoring of axonal damage was higher, although not significantly, in group **B** (1.6±0.9) than in groups **A** and **C** (respectively 0.9±0.6 and 1.0±0.5). The result is consistent with a previously reported lack of association between histological and functional outcome measures of nerve regeneration.[10]

Conclusions

Direct exposure of a peripheral nerve to Piezosurgery®, even in worst case scenarios, did not dissect the nerve, but induced structural and functional damage. Consistently with a previous study,[5] even following nerve contact at *peak force*, the perineurium of the nerve was intact, and thus the potential for functional recovery was large. Importantly, the extent of damage was significantly higher with increased force applied by the device on the nerve, but not by activation of the ultrasonic vibration. Since a correct use of Piezosurgery® prescribes application of a limited pressure, the safety margins would thus be larger than using instruments which are typically operated at higher force (e.g., bur) or which are likely to cut the nerve upon direct contact (e.g., oscillating saw). This makes Piezosurgery® a promising tool to perform osteotomies in close proximity to a nerve in maxillofacial (e.g., orthognathic surgery of the mandible) or orthopaedic surgery (e.g., laminotomies in the cervical spine).

Acknowledgments

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Legend to Figures

Figure 1

Motor (**a**) and sensory (**b**) nerve damage in animals treated by Piezosurgery® at *working force* for 5 sec without (Group **A**) or with (Group **B**) ultrasonic activation, or *peak force* for 1 sec with ultrasonic activation (Group **C**). See Methods for definition of *working force* and *peak force*.

Figure 2

Longitudinal sections through the sciatic nerves of treated rats after full sensory and motor recovery. The fields are representative of (**a**) isolated axonal damage in the nerve bundle or (**b**) abundant axonal breakdown and segmentation of myelin into digestion chambers together with mild inflammation. Hematoxilin/Eosin stains, bar = 100 μm.

Figure 1

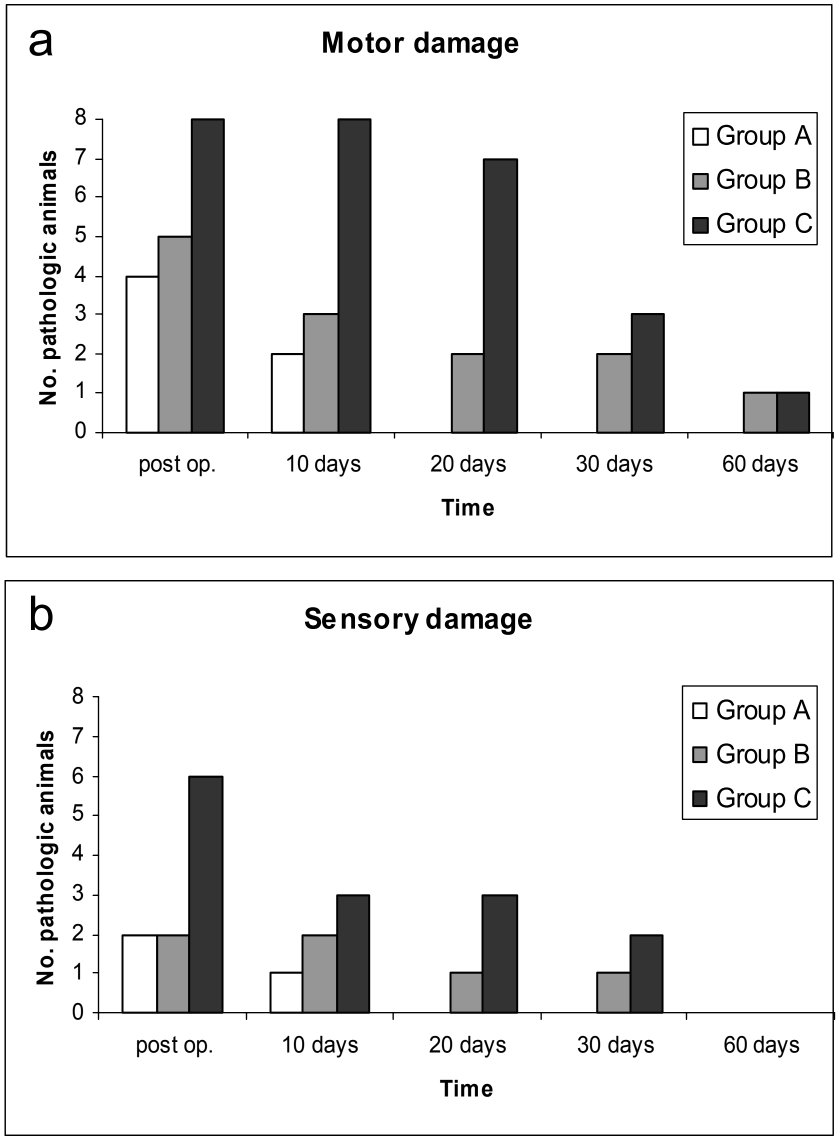
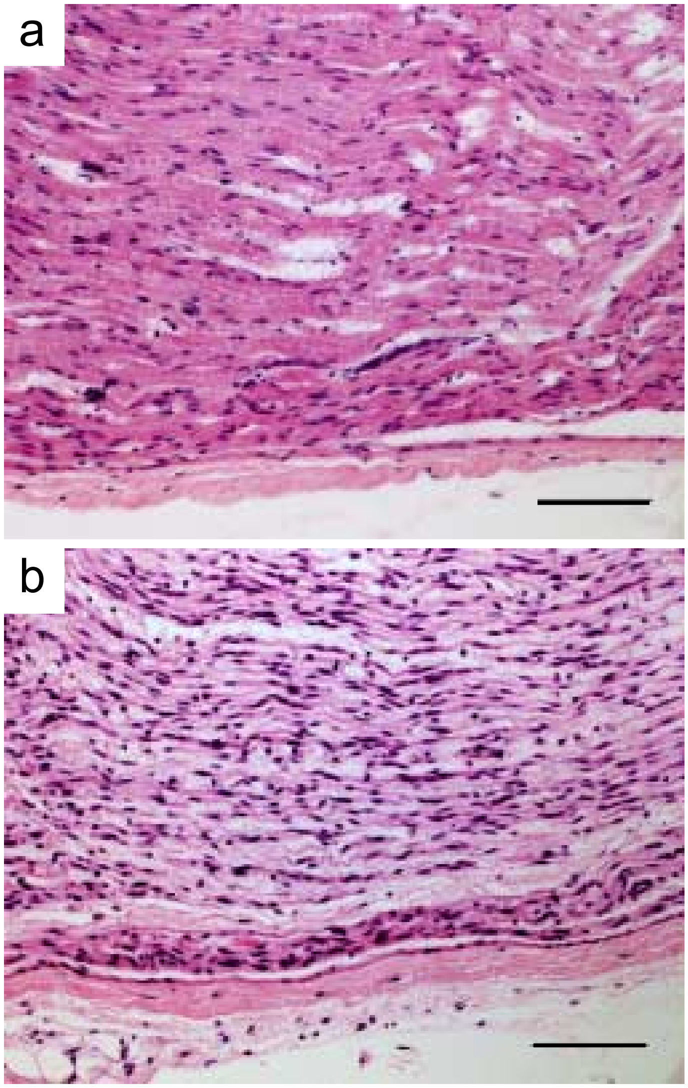

a
Motor damage
No. pathologic animals
0
1
2
3
4
5
6
7
8
Group A
Group B
Group C
post op.
10 days
20 days
30 days
60 days
Time
b
Sensory damage
No. pathologic animals
0
1
2
3
4
5
6
7
8
Group A
Group B
Group C
post op.
10 days
20 days
30 days
60 days
Time

Figure 2



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Disclosure Statement

Publication: *Journal of Oral and Maxillofacial Surgery*

Author: [illegible]

Article Title: Assessment of Nerve Damage using a novel ultrasonic device for cutting bone

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Author: IVAN MARTIN

Article Title: ASSESSMENT OF NERVE DAMAGE USING A NOVEL ULTRASONIC DEVICE FOR CUTTING BONE

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Publication: *Journal of Oral and Maxillofacial Surgery*

Author: Stefan Schaeren

Article Title: Assessment of Nerve Damage using a novel Ultrasonic Device for Cutting Bone

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Author: MARKUS TOLNAY

Article Title: ASSESSMENT OF NERVE DAMAGE USING A NOVEL ULTRASONIC DEVICE FOR CUTTING BONE

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Signature: Prof. Dr. Markus Tolnay

Date: 29/6/06

American Association of Oral and Maxillofacial Surgeons
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Author: TOMASO VERCELLOTTI

Article Title: ASSESSMENT OF NERVE DAMAGE USING A NOVEL ULTRASONIC DEVICE FOR BONE CUTTING

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