

Construction of a standard test assembly for controlled laser studies in tissues: Preliminary study on human bone material

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The aim of the study is the construction of a test assembly, which facilitates objective, comparative studies on the cutting performance of lasers in hard tissue. To ensure the applicability of our own construction for the reproducible performance of laser incisions in hard tissue, eleven freshly extracted blocks ($2 \times 1.5 \text{ cm}^2$) of human bone were prepared with a Er,Cr:YSGG laser by using a handheld handpiece, respectively, using the constructed device for a standardized cutting. A total of 44 cuts were executed. The specimen were then histologically evaluated. The standard test assembly met the requirements concerning the provision of objective results. The findings of the histological evaluation prove the reproducibility of the results. The standard test assembly presented in this paper facilitates comparative studies of different laser systems by reducing subjective influence on the preparation to a minimum. The results of this preliminary study show that the precision of the guiding instrument for laser cutting reduces the error of cut width by 50-fold, from 50 to $1 \text{ }\mu\text{m}$.
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I. INTRODUCTION

Since the late 1960's different laser wavelengths based on numerous studies were established as standard therapy in almost all indication fields of dentistry. During the last years lasers have been used increasingly also in implantology. Due to their different absorption maxima and thermal absorption coefficients, different lasers are considered to be suitable for soft and hard tissues or the application in contact with implant surfaces. According to previous studies only the laser types Er:YAG (YAG denotes yttrium aluminum garnet, Refs. 1–3) and Er,Cr:YSGG (YSGG denotes yttrium scandium gallium garnet)^{4–14} are currently recommended for use in bone and dental hard tissues. Their absorption maxima lie within the H₂O band. By means of a continuously adjustable H₂O and air supply, these devices ensure a sufficient cooling of tissue. Clinical experiences in the use in bone harvesting, implant bed preparation, sinus floor elevation as described by Tatum, and root-end resection are documented.^{15–17,10,18–21} The results of the said studies are comparable only conditionally, as the majority of the studies describes the power settings of the lasers used in each case, but not the constant time and operation distance settings for the examined specimens. Further features arise from the handling of the laser handpiece by the examiner. Since this is a very individual performance and rather difficult to reproduce, its comparability is quite limited.^{15,22,17,10,18,20,22} Therefore the present study deals with the construction of a test assembly to facilitate objective comparable studies on the effects of different laser wavelengths and their work parameters in clinical practice (W, mJ, Hz, pulse duration, pulse pause, and fluency).

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II. MATERIALS AND METHODS

A. Collection of unfixed human bone

For the realization of this study, human bone was collected from unfixed human corpses for 48 h postmortem: mandibula (retromolar area to molar area), interforaminal area (regio mentalis), and maxilla (region tooth 3–5 buccal). The bone was collected with a Lindemann cutter (Company Meisinger, handpiece W&H 30:1 and a transportable OP motor unit of W&H, type 9925SPS) while cooling with 0.9% NaCl solution (liquid/air cooling by a spray nozzle from the dental unit). The collected specimen were taken to the test location in a closed, humid container (cellulose dampened with 0.9% NaCl), cooled with ice water and treated within 6 h according to the presented test assembly under room temperature (24 °C). The bone blocks were trimmed to a size of $2 \times 1.5 \times 1 \text{ cm}^3$. The number of samples cut with laser on the new device was 28, created in seven different bone blocks, giving 7 samples per cutting velocity. A total of 16 cuts were made with the handheld laser handpiece, created in four different bone blocks, giving four samples per velocity.

B. Test assembly 1

The distance of the handpiece to the bone that is defined to be treated could be exactly adhered into this assembly (focus 3 mm), but the handling of the instrument is very individual; the constant laser firing is not comparable in time/distance. Furthermore, the later expressed recommendation of the manufacturer (Biolase Technologies) to keep a beam angle of 25° (to protect the damageable sapphire tip) was not workable with this construction. The gathered specimens showed unsatisfying, individual results. Though it is



FIG. 1. (Color online) Constructed and fixed calibration unit on the laser handpiece to be used (total view). Handpiece, angle 90° (Millennium Waterlase, Biolase Technologies, San Clemente, CA, USA).

possible to perform a histological examination, it does not produce comparative, reproducible results (Fig. 1).

C. Freehand trial for the comparability with other clinical experiments

In four bone blocks the laser cuts were executed with the handpiece used freehand by the examiner, performed to facilitate a relation with the clinical experiments cited in the Introduction (series of specimens 1/0, 2/0, 3/0, and 4/0). Sapphire tip Collonna, G 6; contra-angle handpiece 90° standard; 3.5 W. Four cuts per block were done with different velocities, trying to stick to a velocity of 1 mm/2 s, 1 mm/3 s, 1 mm/4 s, and 1 mm/5 s. Thus overall 16 hand-held cuts were done. The widths of the cuts done by free hand preparation in the study were measured to be 1 mm (± 0.05 mm).

D. Test assembly 2

Since neither experimental assemblies for keeping the exact distance, nor the correlation of defined removal patterns with the speed of the passage over different types of tissue were described, there are no corresponding test assemblies to be found in the literatures. To be able to observe both the factor “local temporal exposure” and the required beam angle to the specimen, the following technical requirements were set up for the test assembly.

- The specimen must be adjustable on the object table in x and y coordinates. The z axis is standardized by a spacer; an optical distance control for an uneven surface might also be possible.
- The operation cannot be carried out freehand. It has to be performed using stepper motors and be absolutely reproducible. The movements of the preparation table and the operation by stepper motors have to enable a consistent movement of the specimen, meaning that the motion over the x and y axes has to be smooth and without jolts. The control programs certainly have to be able to imitate possible temporal movements of the examiner.
- A computer must be used as controller, transmitting variable control programs to the stepper motors.

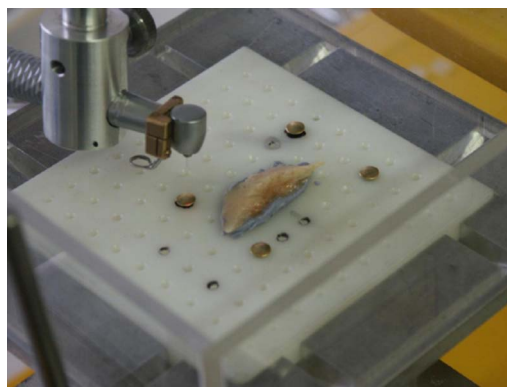


FIG. 2. (Color online) Technical configuration for the determination of the reference field and the reference point.

- Additional water and air supply shall be available as extension of the test assembly.
- Suctioning of present liquids.
- The mounting of different handpieces of various lasers for comparative studies should be easy to carry out.
- It should be possible to control the coupling holder (mounting device) of the laser handpiece using a stepper motor later on, to be able to modify the heights on the z axis.

E. Lasers and technical specifications used in the study

- Biolase Millennium, (Biolase Technology Inc., San Clemente, California, USA);
- medium: Er, Cr:YSGG (Erbium, Chromium, Yttrium, Scandium, Gadolinium Garnet);
- wavelength: 2780 nm, laser class: IV;
- frequency: 20 Hz;
- preciseness of output: $\pm 20\%$ (application of a standardized measuring device during the trials); following Ref. 23, item 50.2;
- single pulse energy: 0–300 mJ;
- pulse duration: 140–150 μ s;
- handpiece angle (standard): 90°;
- sapphire tip diameter: 200–750 μ m (here, sapphire tip: type G 6, Colonna-Tip);
- distance from tissue: 3 mm ($\pm 15\%$), angle position 25%;
- beam expansion: 8%;
- pilot beam: laser diode, laser class I, 655 nm;
- water and air supply: distilled or sterile water;
- droplet size: 5–200 μ m;
- maximum droplet speed: 100 m/s;
- external air pressure supply: 5.5–8.2 bars (80–120 psi);
- range: 0.5–8.0 mm in front of the handpiece tip (depending on the power, water, and air settings on the device);
- power settings in this study: 3.5 and 5 W.

F. Reference setting

The exact reference setting of the laser handpiece was realized by means of the integrated pilot beam of the laser

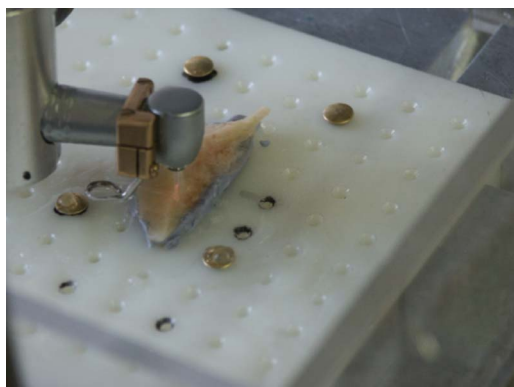


FIG. 3. (Color online) Laser in operation mode above embedded bone preparation; red color means pilot beam.

(laser diode, laser class 1,635 mm) using a control program (Fig. 2).

G. Preparation and embedding of the donor bone on the preparation table

To eliminate different penetration depths and possible differences in tissue ablation, the gathered bone material was planed in the working plane while cooling with NaCl 0.9% using a cross-toothed bone burr (Company Meisinger, handpiece W&H 30:1 and a transportable OP motor unit of W&H, type 9925SPS). The bone block was pressed into a slowly hardening silicone impression compound with a punched teflon panel as basis and adjusted in the horizontal and vertical levels. Arising humidity was removed during the whole trial by means of a strong suction in direction of the preparation table.

H. Alignment of the laser handpiece

First the working angle of the laser (25°) was adjusted at the height support with mounted angle adjusting device and fixed above the reference point. The working height above the bone was adjusted only when the bone was embedded by means of the reference program. The respective bone was embedded in a way that the reference point was located on the same level with the first preparation section. With the specimen aligned in this manner, the first step of the procedure could be performed starting from the reference point above the preparation in the respective individual control operation (see Fig. 3). The laser handpiece was mounted statically in an angle of 25° (protection of the preparation tip against flexion and destruction). The height was adjusted on the basis of the position of the embedded piece of bone.

I. Selection of the operating program and realization

For the realization of the preparation, the corresponding “job” was selected and the operation program for the respective trial was started. The following is an example of the description of the performed operation programs: PCNC1-sequence “24 mm_um3.job.” The table with the preparation runs in the horizontal direction over a distance of 24 mm with a speed of 1 mm/s, and then shifts the direction of

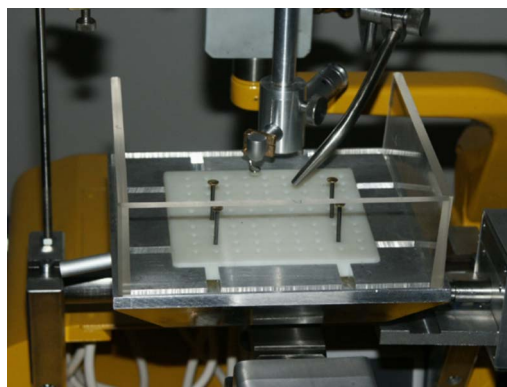


FIG. 4. (Color online) Preparation table with laterally mounted suction attachment and inserted teflon lining for the preparations.

operation to the vertical direction by 3 mm in 30 s. Subsequently the preparation table runs the next job parallel to the axes over 24 mm with a speed of 1 mm/2 s, and then again shifts vertically by 3 mm in 30 s. After completing the program (in this example it is three runs with different speeds) the table returns to the reference point automatically (Fig. 4). Due to the control program exactly the same job could be run with the same preparation twice. See the example in Sec. IV (Fig. 11, preparation B1). This example shows the exact reproducibility of the control program.

J. Mechanical and technical structure

The structure of this test assembly was realized according to the conditions previously set for the test assembly. The sketches for the structure and the necessary additions were forwarded to an engineering company (Fa. Walter Theil, Oldenburg, Germany) and set up in cooperation with the author. The mechanical and technical structure of the trial with mounted stepper motors, without electronic accessories, is presented in the following figures: preparation table, Fig. 4; laser handpiece, Fig. 5; height support, Figs. 6 and 7; and device, Fig. 8.

Electronic control and stepper motors. After preliminary trials with the first written PCNC sequences and the elimination of sources of error in the control of the stepper motors and mechanical transmission errors to the preparation table

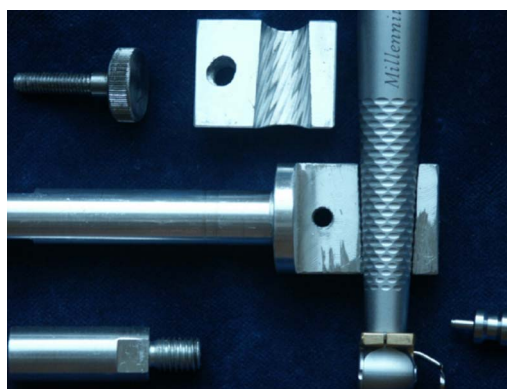


FIG. 5. (Color online) Detail of handpiece in coupling holder with mounted spacer.

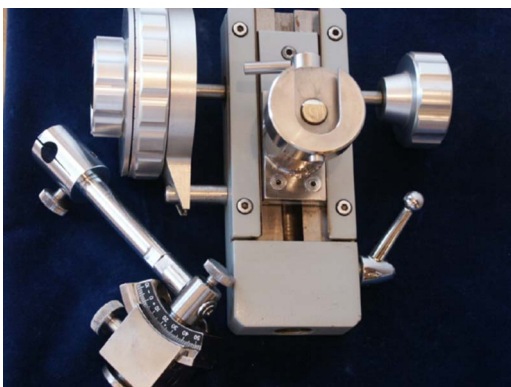


FIG. 6. (Color online) Height support with angular adjusting device for laser handpiece.

unit, the respective operation programs were defined. The “reference job” was defined on the basis of possible movement lengths of the cross support and marked on the preparation table. The fixed stepper motors are connected directly with the spindles of the cross support on the respective axes. Trials with gimbal connections were discarded (slackness of steps in waves working in a gimbal manner). The thread pitch of the cross support is 0.8 mm. The stepper motors are controlled by means of a control card, which in turn receives the commands via the control program of the computer. Electricity is supplied by a direct current power supply with a voltage of 24 V. The stepper motors run with a frequency of 5 steps/mm. The thread pitch of the x and y axes is identical. A 360° rotation moves the preparation table each time by 0.8 mm in the horizontal and vertical directions. The maximal movement unit (movement of the preparation table above the coordinates) was defined as 1 mm/s. At a higher speed no Er:YAG laser in the spectrum of dental applications can bring about a useful and measurable effect. As minimal movement the threshold of 1 mm/7 sec was specified (Fig. 9). The lengths of the tissue covered by the laser in the x and y coordinates were chosen in a way that the different laser parameters could be easily altered during the procedure.

Additional supply of air and water: The actual amount of air in the spray (H_2O/air) could not be recorded. The actual water flow was measured on the handpiece. It amounted to



FIG. 7. (Color online) Angle adjusting device mounted horizontally. The coupling holder for the laser handpiece can be rotated in any direction.



FIG. 8. (Color online) Overview on complete measuring device.

40 ml/min. Transferring to the covered distance of 1 mm/sec consequently, the water amount sprayed on the bone at the setting 65/65 ($\%H_2O/\%air$) equated to the quantity of 0.67 ml/mm. However, this value cannot be converted linearly with the values to be adjusted on the panel. Moreover, several comparative measurements showed deviations of 5%–10%.

III. RESULTS

Evaluation of the reproducibility of the trials by means of histological evaluation of the bone preparations. Following every trial, the bone was removed from the embedding material and subjected to standard preparation for histological examination. Formaldehyde; dehydration in an ascending ethyl alcohol series (50%–100%); methanol bath, embedding in methacrylate; microtome preparation. The bone preparations were subjected to a macroscopic evaluation under $3.5 \times$ loupe magnifying on the one hand, (Figs. 10 and 11) and a histological assessment at the Anatomical Institute of the Munich Ludwig-Maximilians-University, on the other hand, to verify the reproducibility of the cutting movements over the bone. The demand of achieving multiple passes over the tissue without hitting the preparations walls again was reached. Single passes over the tissue with increasing exposition time showed increasing incision depths with uniform collateral damage zones along the incision. The mean collateral damage zone was measured to be 5–15 μm (Fig. 12).



FIG. 9. (Color online) Fully mounted mechanical experimental setup, including electronic control, power supply, and control card.



FIG. 10. (Color online) Macroscopic assessment of sample B2; from left to right: 1 mm/2 s, 1 mm/3 s, and 1 mm/4 s. No carbonization and constant incision depth and width along each preparation line.

The widths of the cuts done by machine driven preparation in the study were measured to be 1 mm (± 0.001 mm), whereas the handheld preparation gave cut widths of 1 mm (± 0.05 mm). The results show an improvement in accuracy of the cut by 50-fold.

IV. DISCUSSION

The trial conditions described in the literature are inconsistent, they often represent clinical experiences and the details are subjective to some extent. To standardize these details of application, a system which makes it possible to assess all different laser systems and compare them with each other was developed. This standardized test assembly facilitates an investigation of the effect of different lasers on hard tissue in comparative studies. Another precondition for the exact reproducibility of incision trials with the laser is the detection of the laser output power directly before and after every use of the device. Only then the effective radiation output of the device can be documented.

In the present study only the effect of the Er:YSGG laser (Biolase) on human bone in connection with constant parameters was documented. Other studies investigated different laser wavelengths such as the CO₂; Ivanenko *et al.* reported that the carbonization zone of bone cuts was 150–500 μm ,



FIG. 11. (Color online) Macroscopic assessment of sample B1, double path with the same program. Note no carbonizations, exact repassing in the same cut, thus only deepening the cut without damage to the incision walls.

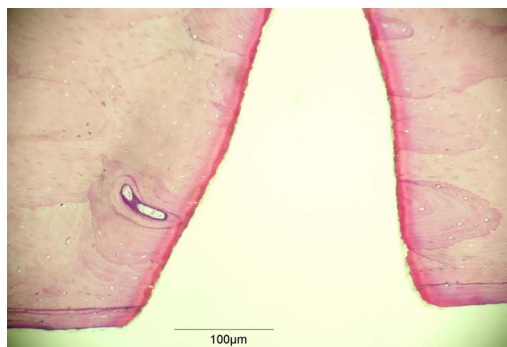


FIG. 12. (Color online) Microscopic assessment of sample B1: no steps or “double hits” in the preparation walls, uniform collateral damage zone of 8 μm .

and even more. Similar or even stronger thermal side effects appear by application of the Nd:YAG and Ho:YAG lasers. The zone of thermal necrosis in cortical tissue after Er:YAG laser incision varies according to different reports from 10 to 50 μm which is comparable with the damage from a mechanical saw.²⁴

Though the technical test assembly is very elaborate in its details, it is precisely this complexity that facilitates a great variability of trial designs. The intended reproducibility was achieved. After numerous preliminary trials, the control system was designed in a way that it enables the examiner to extend it freely based on current standard and equip it with a new programming at any time. The planned stepper motor for a movement of the laser with exactly adjusted height above the object is currently causing technical teething troubles. The difference in separation distance of $\pm 15\%$ (laser tip to object) is a consequence of the horizontal arrangement of the bone on the preparation table by hand. A mechanical eligibility of the cutting level on the block preparation would be helpful.

V. CONCLUSION AND PERSPECTIVE

The results of the test assembly allow us to present this apparatus as standard test assembly. Due to its technical details, as well as parameters that can be defined and selected freely, it is suitable for the realization of the necessary comparative studies with lasers. The standard test assembly presented in this paper facilitates comparative studies of different laser systems by reducing subjective influence on the preparation to a minimum. The results of this preliminary study show that the precision of the guiding instrument for laser cutting reduces the error of cut width by 50-fold, from 50 to 1 μm .

It would also be of great use to perform studies with different lasers on the same embedded tissue. Not only could they be carried out on the same (authentic) piece of tissue, but owing to the spatial positioning of the laser device it is possible to document histological comparative studies also on small amounts of tissue very economically and clearly. In this way comparative laser studies can be accomplished objectively in the future. It would also be possible to perform studies in soft tissue and implants using easily realizable modifications.

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