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ORIGINAL ARTICLE

Temperature and depth evaluation of the in vitro effects of femtosecond laser on oral soft tissue, with or without air-cooling

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Abstract

Femtosecond laser is an effective and safe tool in many surgeries, but the studies of its effect on oral soft tissue ablation are insufficient. This study aimed to investigate the effect of soft tissue ablation with a 1030-nm femtosecond laser on temperature and depth. Twenty Sprague–Dawley rat tongue specimens were obtained and flat-mounted. The 1030-nm femtosecond laser was controlled by a computer system, with a set distance of 4.7 mm between the laser aperture and soft tissue surfaces. Ten specimens were ablated for > 1 min with or without air-cooling for temperature measurement, while the other 10 specimens were ablated for depth measurements, using the following parameters: (i) 3 W, 2000 mm/s; (ii) 3 W, 4000 mm/s; (iii) 5 W, 2000 mm/s; (iv) 5 W, 4000 mm/s; (v) 8 W, 2000 mm/s; (vi) 8 W, 4000 mm/s. Temperature changes were measured using a type-K thermocouple. The depth attained using different power and scanning speed settings was measured by a three-dimensional morphology measurement laser microscope. Laser power, scanning speed, and air-cooling effects were determined. Higher energy and lower speed induced higher temperatures ($p < 0.05$), which were significantly decreased by air-cooling ($p < 0.05$). The lowest ablation depth was obtained at 3 W and 4000 mm/s (72.63 ± 6.47 μm) ($p < 0.05$). The greatest incision depth was achieved at 8 W and 2000 mm/s (696.19 ± 35.37 μm), or 4000 mm/s (681.16 ± 55.65 μm) ($p < 0.05$). The 1030-nm femtosecond laser application demonstrates clinically acceptable ablation efficiency, without marked temperature damage, in a controlled manner.

Keywords Ablation efficiency · Femtosecond laser · Soft tissue · Thermal effect

Introduction

In traditional dental surgery, tissue ablation is often required. Soft tissue incisions are mostly performed using a surgical scalpel; however, techniques employing ultrasound scalpels [1], electrosurgery [2], radiosurgery [3, 4], and lasers of different wavelengths have been reported.

Lasers are now commonly used in oral surgery. Laser devices use the ablation properties of various wavelengths to generate defined ablation lines and are beneficial due to their hemostatic and sterile nature. Additionally, given the small and non-contact operating head, lasers are particularly suitable for use in the tightly confined intraoral environment.

Several different lasers are in use for intraoral soft tissue surgery: CO_2 laser [5], potassium titanyl phosphate (KTP) laser [6], diode laser [7], erbium-doped yttrium aluminum garnet (Er:YAG) laser [8], neodymium-doped yttrium aluminum garnet (Nd:YAG) laser [9], and erbium, chromium-doped yttrium, scandium, gallium and garnet (Er,CR:YSGG) laser [10]. The CO_2 laser has been used for decades, due to its affinity for water, and is frequently used in the treatment of oral mucosa lesions, such as leukoplakia [11]. It has shallow penetration, which is useful for making shallow incisions, such as incisions that need to be made close to a tumor [12]. The CO_2 laser also provides a clean, bloodless surgical field,

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because of its hemostatic and coagulating properties, and causes minimal swelling and scarring [5]. The Er:YAG laser produces less heat and thus promotes rapid healing, and can be safely used in oral biopsy investigations, without affecting histological evaluation, and produces fewer thermal side effects than CO_2 and Nd:YAG lasers [8, 13]. However, its hemostatic effect is not as good as that of the CO_2, Nd:YAG, or diode laser, which are less absorbed by water and more absorbed by hemoglobin and melanin, and thus have a deeper effect on tissues [14]. Diode lasers are light, low-cost lasers; most diode lasers focus on a 810-nm wavelength. Natekar et al. suggested that pain was significantly less after diode than after CO_2 laser surgery [11]. However, reducing collateral thermal damage from diode laser incisions is clinically necessary to promote wound healing [15]. Recently, a blue-light semiconductor laser device with a wavelength of 445 nm was introduced for dental applications. Due to its shorter wavelength, it inhibits bacteria and scatters less; with this more focused light, there is less risk of damaging the surrounding tissue. Its higher absorption coefficient in the target chromophores, such as hemoglobin and melanin, promotes coagulation. With less laser energy absorption from scattering, the thermal side effects should be fewer [16].

Femtosecond laser is applied in many medical fields. The commonest application is in eye surgery, such as corneal ablation [17, 18]. Mencucci et al. reported that the temperature increase at the fragmentation volume base was around 5 °C in vitro, and lens fragmentation performed using the Victus femtosecond laser might be considered safe [19]. Femtosecond laser has also been applied to bone ablation [20, 21], tooth preparation [22, 23], artificial cochlear modification [24], micro-nano processing of materials, including implant surface modification [25], and cardiovascular stenting [26]. The femtosecond laser can also be applied to molecular level transduction [27, 28], and chromosome [29] and organelle ablation [30] in the cell.

The femtosecond laser is also efficacious for ablating skin tissue, such as removing scar and melanin [31, 32] and ablating cartilage [33], stomach and intestinal mucous membranes [34], vocal cord [35], urethra [36], etc. Some studies have shown that, by selecting suitable laser parameters, femtosecond lasers can selectively remove melanin, while reducing the damage in low-melanin areas of the skin [31]. Mod et al. used femtosecond laser instead of mechanical debridement to remove necrotic tissue from burnt skin and for biopsy [32]. Su et al. [33] used two types of femtosecond laser—a wavelength of 1700 nm, repetition rate of 5 kHz, and laser energy of up to 60 μJ/pulse, and a wavelength of 1053 nm, repetition rate of 30 kHz, and pulse energy of 3 μJ/pulse—and successfully ablated cartilage and bone in vitro, with no noticeable carbonization. Choi et al. [34] described a novel endoscopic microsurgery technique for ablating stomach and intestinal mucous membranes in the murine colon, with high spatial and temporal precision. Hoy et al. [35] applied precise femtosecond laser microsurgery to the vocal cord, using a 776-nm pulse and a frequency of 500 Hz. Liang et al. [36] reported femtosecond laser ablation of the urethra and endoscopic treatment of benign ureteral stricture, using bladder specimens from Sprague–Dawley (SD) rats as surrogate models, both in air and in saline.

The incision depth and width, and the area and depth of carbonization, necrosis, and reversible damage correlate strongly with temperature [37]. Power density, pulse repetition rate, pulse duration, overlap rate, and scanning speed influence ablation efficiency, temperature, and biological reactions [38]. Therefore, an optimal combination of different parameters is crucial for reducing the peripheral temperature and enhancing ablation efficiency. However, most of the femtosecond ablation studies to date have focused on precise endoscopic treatment, and the impact of different parameters on changes in ablation temperature and efficiency in oral tissue after automatically controlled femtosecond laser ablation has not been reported widely.

Thus, this study assessed the efficiency of soft tissue ablation with a 1030-nm femtosecond laser in an automated system, and compared thermal change and ablation efficiency associated with different parameters, to lay the foundation for development of an automatic, femtosecond laser oral surgery robot.

Material and methods

Animal sources

All SD male rats were from Peking University Health Science Department, with weight ranging from 180 to 220 g.

Ethical approval

The protocols involving animals were approved by the Biomedical Ethics Committee of Peking University (protocol no. LA2017281). All animals were raised under standard conditions and were treated according to the National Institutes of Health Laboratory Animal Care and Use Guidelines.

Laser system setup

Six different parameters were used for this study. The femtosecond laser was a fiber laser (TANGERINE, Amplitude Systemes, Bordeaux, France). The output laser beam used in the experiment had a wavelength of 1030 nm, repetition rate of 200 kHz, and laser energy of up to 100 μJ/pulse.

Ablation process for temperature measurements

All measurements were performed under controlled humidity (20%) and temperature (24.5 °C) in the operative room.

For in vitro temperature monitoring, 10 SD rat tongues from 10 SD rats were used, immediately after slaughter. The samples were ablated with automated femtosecond laser under conditions in which parameter settings were varied to determine the temperature change. Three-millimeter linear incisions were made, under computer control. The samples were divided into 12 groups randomly. The samples with no air-cooling were ablated using six different conditions, (groups 1, 3, 5, 7, 9, 11), and the samples with air-cooling were ablated using these six conditions (groups 2, 4, 6, 8, 10, 12). Ablation in each group was repeated five times, making a total of 60 incisions. The power settings were 3, 5, or 8 W, and the scanning rate was 2000 mm/s or 4000 mm/s. The conditions were as follows:

Group 1: power, 3 W; scanning speed, 2000 mm/s; no air-cooling ($N = 5$, 8.3%)
Group 2: power, 3 W; scanning speed, 2000 mm/s; air-cooling ($N = 5$, 8.3%)
Group 3: power, 3 W; scanning speed, 4000 mm/s; no air-cooling ($N = 5$, 8.3%)
Group 4: power, 3 W; scanning speed, 4000 mm/s; air-cooling ($N = 5$, 8.3%)
Group 5: power, 5 W; scanning speed, 2000 mm/s; no air-cooling ($N = 5$, 8.3%)
Group 6: power, 5 W; scanning speed, 2000 mm/s; air-cooling ($N = 5$, 8.3%)
Group 7: power, 5 W; scanning speed, 4000 mm/s; no air-cooling ($N = 5$, 8.3%)
Group 8: power, 5 W; scanning speed, 4000 mm/s; air-cooling ($N = 5$, 8.3%)
Group 9: power, 8 W; scanning speed, 2000 mm/s; no air-cooling ($N = 5$, 8.3%)
Group 10: power, 8 W; scanning speed, 2000 mm/s; air-cooling ($N = 5$, 8.3%)
Group 11: power, 8 W; scanning speed, 4000 mm/s; no air-cooling ($N = 5$, 8.3%)
Group 12: power, 8 W; scanning speed 4000 mm/s; air-cooling (N = 5,8.3%)

Ablation process for incision depth measurement

For depth measurement, the tongues of 10 SD rats were used, immediately after slaughter. Six incisions were made in each tongue, using six different group conditions. Every incision was a 3-mm line controlled by the computer system, and the number of ablations used for each incision was 2000. Each ablation time was recorded by a stopwatch (SP17XL-009A, Fuzhou Swell Electronic Co., Ltd., Fu-jian, China). Each condition was repeated 10 times, for a total of 60 incisions. The groups were as follows:

Group 1: power, 3 W; scanning speed, 2000 mm/s ($N = 10$, 16.6%)
Group 2: power, 3 W; scanning speed, 4000 mm/s ($N = 10$, 16.6%)
Group 3: power, 5 W; scanning speed, 2000 mm/s ($N = 10$, 16.6%)
Group 4: power, 5 W; scanning speed, 4000 mm/s ($N = 10$, 16.6%)
Group 5: power, 8 W; scanning speed, 2000 mm/s ($N = 10$, 16.6%)
Group 6: power, 8 W; scanning speed, 4000 mm/s ($N = 10$, 16.6%)

Intraoperative temperature measurement

The intraoperative temperature of the surrounding tissue was measured using a Data Logger Thermometer (TR-176C, Yuqing Technology, Co., Ltd., Taipei, Taiwan), with a K thermocouple probe. The measurement error was 0.3 °C. The temperature was measured in a continuous manner and the thermocouple recorded temperature once per second. To ensure maximum comparability and a highly standardized procedure for the 10 samples, the thermocouple was located 1 mm away from the ablating area, with the tip of the probe at a depth of 1 mm, and was fixed with adhesive tape to reduce artificial errors. The temperature increase ($\triangle T$) after more than 60 s of laser irradiation was recorded. We used 30 continuous readings from each sample when the temperature had stabilized and repeated the experiment five times.

Depth measurements

After making the six incisions in one specimen, the soft tissue incision depths were assessed by means of a three-dimensional morphology measurement laser microscope (VK-X200, Keyence, Osaka, Japan) and the respective surface analysis software (Keyence) (Fig. 1). The tissue was carefully dried with compressed air. Scanning was performed at a magnification of × 10 with a 16-bit high dynamic range resolution. Slant rectification was added to ensure that the right and left marginal ridge were at the same height. The incision depth was measured between the lowest point and the average distance to the right and left marginal ridge; we chose three values from each incision (Fig. 2).

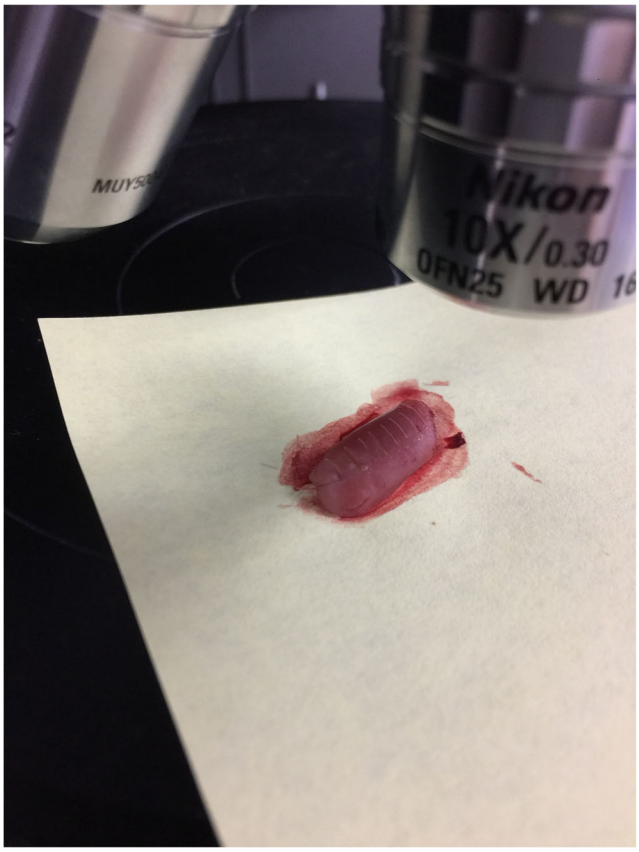

Fig. 1 The specimen after ablation using the six conditions

Statistical analysis

Statistical analyses were performed using the SPSS 19.0 Software Package (SPSS Inc., Chicago, IL). The experimental values were expressed as mean values ± standard deviation. Median and interquartile ranges were calculated when data were non-normally distributed. One-way analysis of variance (ANOVA) was performed to determine the differences between groups for each evaluated parameter. Non-parametrical tests (κ independent sample tests, Kruskal–Wallis test) were used when equal variances were not assumed in one-way ANOVA. The level of significance was defined as $p < 0.05$.

Results

Temperature evaluation

Our data suggested that, as the laser started radiating, the temperature rose and stabilized within less than 10 s (Fig. 3). As the temperature increases in the 12 groups were non-normally distributed ($p < 0.05$), data are presented as median and interquartile range. The highest temperature increases were seen in groups 9 (32.25 ± 4.82 °C) and 11 (31.40 ± 4.62 °C), while the lowest temperature increases were seen in group 4 (6.00 ± 1.88 °C) (Table 1). Comparisons of groups 1 and 2, 3 and 4, 5 and 6, 7 and 8, 9 and 10, and 11 and 12 revealed that air-cooling resulted in markedly lower temperature increases ($p < 0.05$) (Fig. 4). Comparisons of groups 1, 5, and 9; 2, 6, and 10; 3, 7, and 11; and 4, 8, and 12 demonstrated that decreased power produced lower temperatures ($p < 0.05$). In comparisons of groups with different scanning speed settings, some groups, including groups 1 and 3, 2 and 4, 6 and 8, and 10 and 12, showed statistically significant differences ($p < 0.05$), while groups 5 and 7, and 9 and 11 did not ($p > 0.05$), suggesting that scanning speed only slightly influenced temperature changes.

Incision depth evaluation

One-way analysis of variance was used to compare different groups. The average ablating time for a scanning speed of 2000 mm/s was 15.74 s, while the average ablating time for a scanning speed of 4000 mm/s was 12.37 s. The estimated times required to make an incision with a depth of 1 mm were calculated in each group; group 2 took the longest time (170.31 s), while group 6 took the shortest time (18.16 s) (Table 2). The incision depth comparison showed a significant difference depending on the treatment group. Ablation depth among groups 1 and 2 did not reach a depth of 200 μm with 2000 repeats, while groups 5 and 6 had significantly greater depths. The lowest incision depth was obtained with a power of 3 W and a scanning speed of 4000 mm/s (group 2; 72.63 ± 6.47 μm) ($p < 0.05$). The greatest incision depth was achieved in groups 5 (696.20 ± 35.37 μm) and 6 (681.16 ± 55.65 μm) ($p < 0.05$) (Table 3).

By comparing different power groups, we found that groups 1, 3, and 5 and groups 2, 4, and 6 differed significantly ($p < 0.05$) (Fig. 5), revealing the impact of power on ablation depth. Additionally, groups 1 and 2 and groups 3 and 4, with different scanning speeds, differed significantly. However, although there was a slight difference in the mean values of groups 5 and 6, it did not reach statistical significance ($p > 0.05$).

Discussion

The present study showed the temperature changes and incision depths in soft tissue using femtosecond laser with different ablation parameters. We show that femtosecond laser can be operated within certain parameter settings and with air-cooling to achieve surgical incisions effectively.

The ablation speed is an important factor in the efficiency of laser devices. The scanning speed we chose was based on our previous bone and teeth ablation experiments, which showed a good overlap rate and relatively smooth incision.

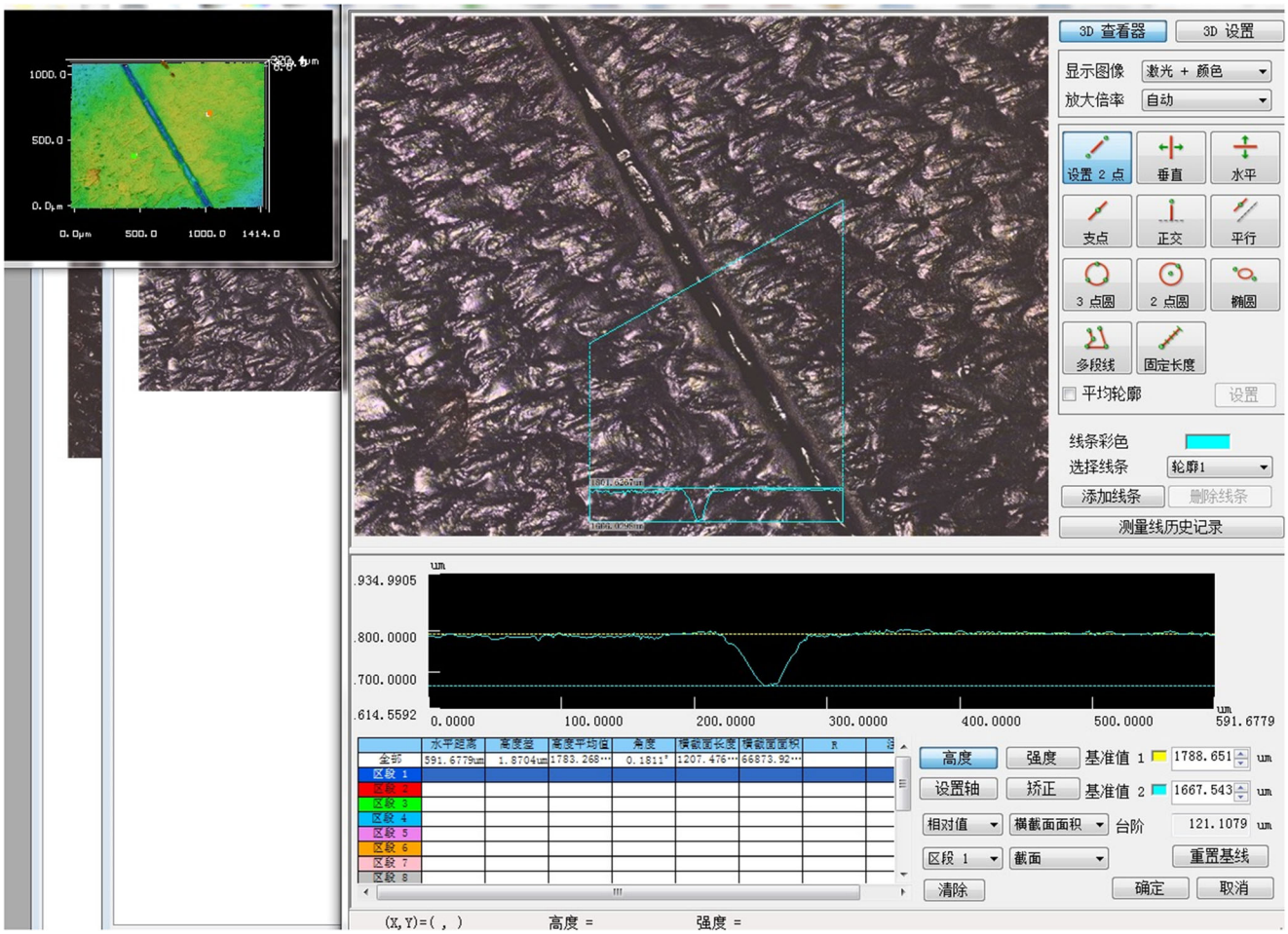

Fig. 2 Three-dimensional profile of a Sprague–Dawley rat tongue specimen. A three-dimensional morphology measurement laser microscope (VK-X200, Keyence, Osaka, Japan) was employed for ablation

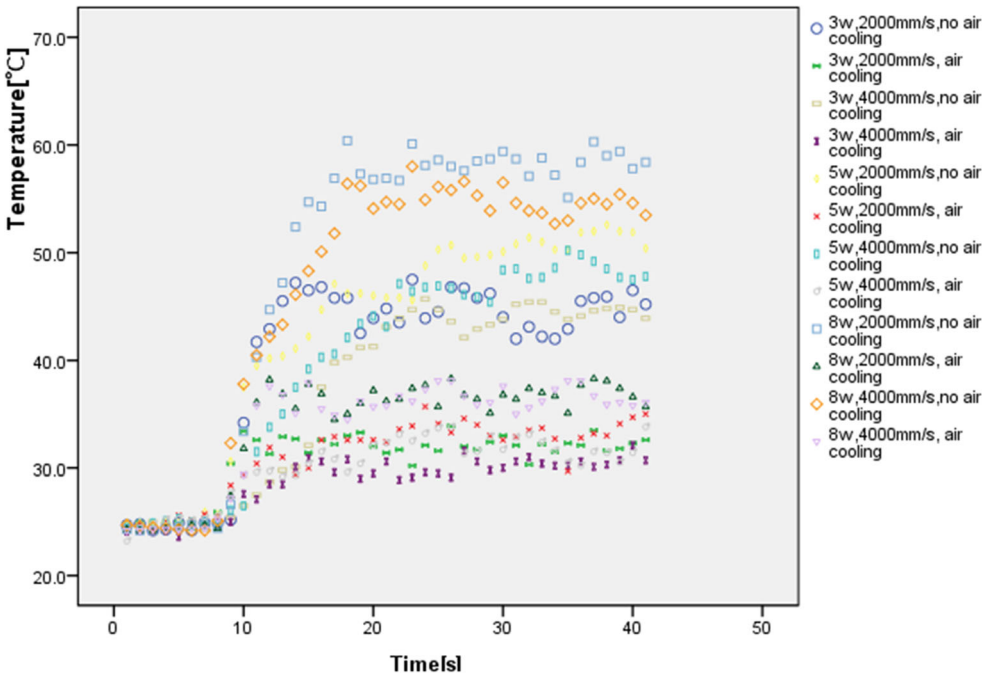

Fig. 3 Temperature as a function of the process time

Table 1 Temperature changes in the 12 groups

Group	Median (°C)	Interquartile range
1	21.00	2.20
2	8.10	2.70
3	20.50	2.35
4	6.00	1.88
5	25.10	1.90
6	10.25	2.70
7	24.40	3.40
8	7.80	1.90
9	32.25	4.82
10	12.90	3.65
11	31.40	4.62
12	12.40	2.23

Table 2 Average time needed for achieving an incision depth of 1 mm

Group	Ablating time/1 mm (s)
3 W, 2000 mm/s	143.67
3 W, 4000 mm/s	170.31
5 W, 2000 mm/s	38.63
5 W, 4000 mm/s	36.03
8 W, 2000 mm/s	22.61
8 W, 4000 mm/s	18.16

Here, we showed increased incision depth and total thermal damage width with decreasing speed; a better quality incision and the lowest temperature increase was achieved with increasing speed. Although, in our study, some groups with different scanning speeds did not differ significantly, it was clear that laser power and scanning speed had a statistically significant impact on the results.

Using a power of less than 3 W or greater than 8 W is not advisable for soft tissue ablation, as we have previously shown that a power of less than 3 W could remove tissue, but had a low ablation rate: it took more than 1 min to produce a macroscopic incision and more than 2 min to ablate to a 1-mm depth. On the other hand, using a power of more than 8 W will be too rapid and dangerous; for instance, using a power of 10 W could ablate a rat tongue completely within 5 s.

In our study, air-cooling significantly decreased the temperature and played an important role in femtosecond laser ablating; without air-cooling, even the mildest temperature increase exceeds 20 °C, while with air-cooling, there is less carbonization and burnt smell, and the highest temperature increase is only around 12.9 °C. Air-cooling can also inhibit inflammation. Uncooled laser wounds, but not precooled laser wounds, have been associated with significantly greater levels of immediate mast cell degranulation than scalpel wounds [39].

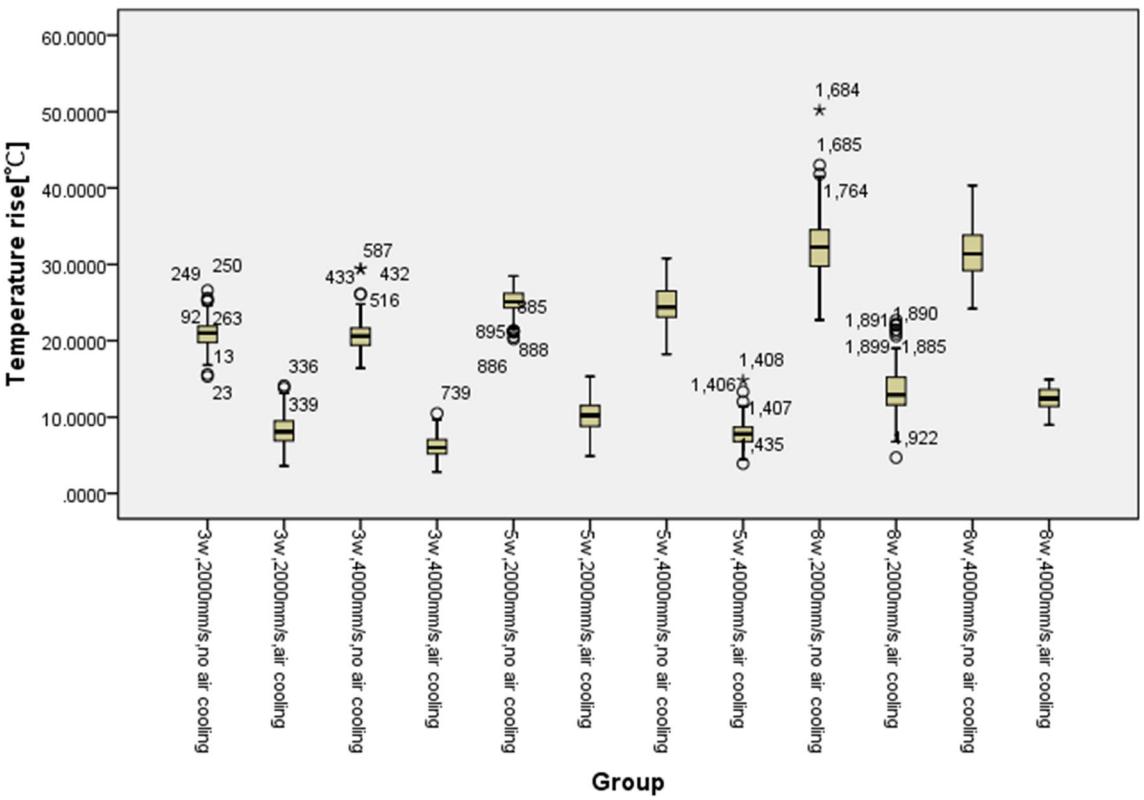

Fig. 4 Box plot of temperature increase when using different parameter settings, with or without air-cooling

Table 3 Mean and standard deviation of ablation depth using six different conditions

Parameters	Mean depth (μm)	Standard deviation	*p* value
3 W, 2000 mm/s	109.56	24.93	0.548
3 W, 4000 mm/s	72.63	6.47	0.520
5 W, 2000 mm/s	407.47	38.20	0.204
5 W, 4000 mm/s	343.33	28.47	0.668
8 W, 2000 mm/s	696.20	35.37	0.868
8 W, 4000 mm/s	681.16	55.65	0.078

The focal distance is also very important. When the optimal range (in our case, around 4.7 mm) was exceeded, the light would turn from white to green, and the ablation efficiency reduced markedly; even with the highest power, it required a long time to ablate the tissue and generated much carbonization and tissue denaturation. However, by acquiring the average incision depth for each layer and setting this in the computer system, the focal plane could be stepped forward, following the incision depth, to ensure a stable focal distance when deeper incisions are required.

The safe distribution and range of heat in tissues is a crucial factor when ablating bone or soft tissue with high-intensity lasers. The appropriate temperature increase promotes wound healing. According to Capon et al., a 45–50 °C temperature gradient induced by laser in the surrounding skin can induce a heat-shock response (HSR), involving temporary changes in cellular metabolism. These changes are characterized by the production of heat-shock proteins (HSP). HSP70 plays a role in coordinated expression of other growth factors, such as transforming growth factor (TGF)-beta, which is a key element in the inflammatory response and the fibrogenic process [40]. Thermal laser irradiation induced the modification of growth factor synthesis, indicating that appropriate thermal lasers could effectively promote skin wound healing, if they are used in a controlled manner [41]. However, too much heat will generate irreversible damage. According to an in vivo study presented by Augustin et al. [42], the cellular death caused by heat is immediately evident at temperatures above 70 °C. Eriksson et al. [43] reported that, in rabbit tibia, heating to 50 °C for 1 min induces signs of vascular injury, while heating to 60 °C results in permanent cessation of blood flow in bone. According to Hambleton et al. [44], the solubility and susceptibility of collagen to digestion by proteolytic enzymes are increased with temperature. Above 52 °C, there is a sudden increase in both characteristics, suggesting that this temperature is associated with changes in the structure of skin

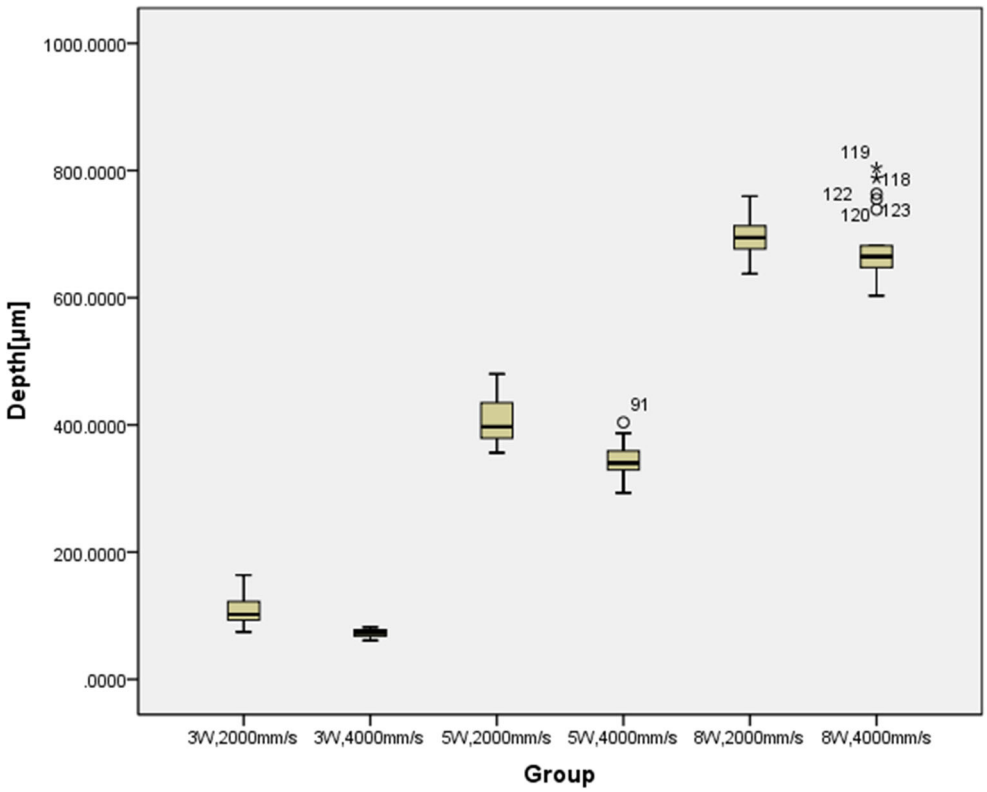

Fig. 5 Box plot of incision depths of 10 rat tongue specimens for different parameter settings

collagen. The increased susceptibility to collagen digestion may affect the healing process in the burn wound. Therefore, the temperature increase in vivo should be less than 15 °C. Without air-cooling, the temperature increase for every condition tested in the present study exceeded 20 °C, with the smallest increase seen in group 3 (3 W, 4000 mm/s, no air-cooling). However, with air-cooling, the mean temperature gradient measured for all experiments decreased markedly; all were in the ideal temperature range, with the highest increase of 12.9 °C seen in group 10 (8 W, 2000 mm/s, air-cooling). This suggested that, with air-cooling, the temperature side effects would be acceptable for all these conditions.

Incision depth is proportional to power; in groups using 3 W, the depth was less than 200 μm with 2000 repeats, the depth in groups using 5 W were around 400 μm, while the depth significantly increased at 8 W: the depth was 696.1962 ± 35.3717 μm in group 5 (8 W, 2000 mm/s), and 681.1613 ± 55.6513 μm in group 6 (8 W, 4000 mm/s). The six parameters discussed in this study all allow ablation of a SD rat tongue in an acceptable time. We estimated the time required for an incision of the same depth: group 6 (8 W, 4000 mm/s) took the least time, while temperature increases were less than those seen in group 5 (8 W, 2000 mm/s), suggesting that it might be a comparably safer and more efficient parameter in a clinical context. Other parameters were also in the safe temperature range when used with air-cooling, but the operation duration might be longer; thus, whether to use a lower or higher power depends on the surgical site and the precision required.

The depth measurements demonstrated relatively large deviations, which indicated that soft tissue ablation was not as accurate as bone or tooth ablation. There were several reasons: first, due to the Gaussian profile property of the laser beam, the fluences varied from pulse to pulse, the soft tissue ablation depth can be varied significantly by changing fluence, in a short radiating time. Moreover, tissues from different organs have different texture and relatively different compositions, such as water content; the same tissue may even have a different texture, depending on the time since slaughter, and thus, the ablation rate may also differ. In the clinic, when using femtosecond laser to ablate oral soft tissue, it is essential that the doctor monitor the process.

Femtosecond laser has many advantages over other lasers because of its characteristics, such as high repetition, short duration, and high instantaneous power. Firstly, they have better precision, because of the small diameter of the light spot; they can even focus on a single cell [27, 34]. Heat is also an influential factor in precision: too much heat leads to carbonization and denaturation [9]; femtosecond laser has relatively high repetition rates, with a short pulse duration; the ablation happens in a very short time. Therefore, the high-intensity energy is limited to a micro-region, and there is insufficient time for heat conduction; the tissue is vaporized, forming laser-produced plasma that removes the heat, thus avoiding significant heat deposition [35], which is especially important for wound healing and esthetics. Secondly, these lasers can ablate both hard tissue, such as bone [20] and teeth [22], and soft tissue [35] due to the wavelength and high-intensity power, and thus they have a wider indication. Other lasers, such as diode laser [14] and KTP laser [6], are used for soft tissue ablation, although CO_2 and Er:YAG lasers [45] can also ablate the bone; however, because the wavelength of the CO_2 laser more closely approximates the absorption peak of water, nearly all of the energy is absorbed in the superficial tissue layer; thus, they are not useful for deep lesions [12]. Lastly, unlike lasers such as the CO_2 laser and the diode laser, which are held in a handpiece, the femtosecond laser is controlled automatically by a computer system; this decreases the artificial errors and labor and allows design of the ablation route before surgery and prediction of the ablation effect.

In conclusion, we compared temperature changes and ablation efficiency for soft tissue incisions using femtosecond laser with different parameters. With air-cooling, all conditions were effective and below the temperature limit of tissue damage: 3 W power yielded comparably lower temperature increases, but much slower ablation, while use of 5 W and 8 W increased ablation efficiency significantly, without increasing thermal side effects markedly. We showed that 1030-nm fiber lasers might be very useful in oral surgery, since they provided acceptable ablation efficiency and relatively low thermal side effects. As this was an in vitro study, further in vivo animal experiments and histological observations of oral epithelium should be performed to assess tissue reactions after making incisions with a femtosecond laser under different parameters.

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Compliance with ethical standards

Conflicts of interest The authors declare that they have no conflict of interest.

Ethical approval The protocols involving animals were approved by the Biomedical Ethics Committee of Peking University (protocol no. LA2017281). All applicable international, national, and/or institutional guidelines for the care and use of animals were followed.

Informed consent Informed consent was not required for this type of study.

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