Application of ultra-fast laser radiation for processing of biological tissue: mass spectrometry of ablated species

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Abstract. In recent years, due to the demonstrated high accuracy and reproducibility of the surgical procedures with fs pulses, the potential of using fs laser sources as a conventional tool for many surgical applications has increased. Moreover, the laser parameters can be so chosen as to modify the tissue in different ways. Future use of lasers in different fields in medicine will be related with the outcome efficiency of the laser treatment procedures. By studying the process of laser tissue interaction we intend to be able to make qualitative improvements to different type of medical applications of lasers. In this study we examined the ablation process of biological samples (tooth and bone) while varying the laser parameters (pulse duration, wavelength, fluence). The mass spectra of organic molecules at $\lambda=193$ nm and $\lambda=800$ nm were compared. We considered the laser intensity effects on the mass distribution of biological samples. The analysis of the ion species produced by short-pulse laser ablation showed that fs laser ablation is more effective than the ns laser ablation. This article provides a description of our understanding of the process of laser-tissue interaction based on mass spectrometry studies.

1. Introduction

Surface laser ablation on the femtosecond time scale has been an important subject of examination for scientists ever since the convenience of utilizing ultra-short laser radiation in many applications in medicine and in material processing was discovered. Femtosecond laser pulses have been used successfully for high-precision middle-ear surgery due to the low thermal and mechanical stress produced, and in ophthalmology for reshaping the human eye cornea [1] and correcting astigmatism and myopia. Dentistry is also an important field of fs laser application [2]. The main efforts have been directed towards preserving the chemical integrity of the tooth surface processed, since conventional methods [3, 4] (dental drilling machines, Er:YAG lasers) show several disadvantages, such as heating of the surrounding tissue, damage of the healthy area, generation of micro cracks in the enamel which are one of the reasons for the development of new caries. Recent studies found that it is possible to use FTIR spectroscopy to characterize laser-induced chemical and crystalline changes after irradiation with a TEA laser operating at 9.6 $\mu$m [5]. It was discovered that under appropriate laser irradiation conditions minor chemical and crystalline changes were only observed in the enamel. These findings showed that after laser ablation the dental tissue is transformed through laser heating into a more acid resistant phase with an enhanced resistance to future decay. There exist several already proven successful procedures in dentistry with ultrashort lasers for treatment of carious lesions and dental
cavity preparation [6]. Laser sealing of dentinal tubules is a new method in dental treatment used to reduce the sensitivity to thermal stimuli. Another important objective in dentistry is cleaning and shaping of the root canal system [7] for elimination of tissue remnants; this procedure is usually performed with hand instruments which leave a smear layer afterwards. By utilizing lasers this disadvantage can be avoided. Recently, femtosecond laser shaping (by ablation) of ceramic restorations [8] yielded promising results in manufacturing of dental crowns and bridges. Ultrafast laser treatment might be an alternative in modern dentistry to conventional methods; by choosing appropriate processing parameters, one can achieve efficient elimination of carious dentine without modification of the surrounding areas. Moreover, the fragmentation process can be controlled by properly selecting the laser energy density, thus influencing the ablation mechanism. The primary advantages of employing ultra-short pulses in dentistry consists in the possibility to achieve better control over the ablation depth per pulse and to reduce the limit for the ablation threshold which drastically influences the ablation process. In particular, “soft” ablation can be carried out for formation of molecular ions of fragile molecules of biological origin.

2. Energy deposition mechanisms

The extremely short duration of femtosecond pulses is the main advantage in tissue processing. With ultra-short pulses the thermal component can be neglected because the pulse duration is smaller than the thermal relaxation time, which for biological material is in the order of 1µs, thus the time for heat distribution is limited and the sample remains relatively cold [9].

Determination of the laser ablation mechanism in femtosecond time-scale is important for understanding the underlying fundamental physics. It was discovered that the mechanism of interaction of the fs radiation with different materials is governed by different laws depending on the material: metals, semiconductors, organic and inorganic compounds. At high intensities, about $10^{13}$-$10^{14}$ W/cm², the electron oscillation energy in the laser electric field is a few electron-volts, which is of the order of magnitude of the ionization potential. Furthermore, at intensities above $10^{14}$ W/cm² the ionization time for a dielectric [10,11] is just a few femtoseconds, typically much shorter than the pulse duration (ca 100 fs). The electrons produced by ionization in dielectrics [12] then dominate the absorption in the same way as the free carriers in metals, and the characteristics of the laser-matter interaction become independent of the initial state of the target. As a result, the first mechanism becomes of major importance for both metals and dielectrics. The ultra-short pulsed laser-matter interaction mode corresponds to conditions when the electron-to-ion energy transfer time and the heat conduction time exceed significantly the pulse duration, $\tau_{ei} \sim \tau_{eh} \gg \tau_p$. Then the absorbed energy is going into electron thermal energy, and the ions remain cold, making the conventional thermal expansion inhibited. However, if the laser intensity is high enough, the electrons can gain energy in excess of the Fermi energy and escape from the target. Hence, an extremely non-equilibrium regime of material ablation takes place. This regime occurs at a laser pulse duration $\tau_p < 200$ fs and at intensities above $10^{13}$-$10^{14}$ W/cm². In that case the escaped electrons accelerate the ions by the electrostatic field of charge separation. For extremely short pulses (<50 fs) multi-photon ionization (MPI) dominates. The absorption of several photons simultaneously leads to ionization of the molecule. Both processes (MPI and avalanche ionization) require a threshold intensity to trigger the optical breakdown.

For pulse lengths of a nanosecond [13] or longer durations the ablation proceeds with heating of the material and melting. In what concerns biological tissues, the process develops with damage of the healthy area, generation of micro cracks, generation of shock waves, formation of cavitation bubbles, micro explosions and mechanical disruption. These effects lead to less control over the shapes and structures. In fact, lasers working in nanosecond or longer ranges show very strong thermal side-effects, they generate too much heat during the ablation process; there is enough time for the thermal wave to propagate and to form a molten layer. In summary the fs interaction with matter can be described as a process of energy transfer to the electrons which usually takes about 10 ps. This time is much longer in comparison with the duration of the fs pulse which leads to minimized heat load to the sample. The research in the area laser ablation of metals and dielectrics has brought about the idea to employ the characteristics of femtosecond pulses in the field of medicine as well.
3. Experimental set-up

The measurements were performed using experimental equipment described earlier [14].

The sample chamber and TOF spectrometer were kept generally at 10⁻⁸ Torr. A primary advantage of the TOF spectrometer is in the possibility for simultaneous registration of the whole mass spectrum of the ablated species. Ion extraction and acceleration is achieved using an ion optic arrangement designed for this instrument. Electrostatic potentials of +2 kV are applied to the ion optics to enable ion extraction.

![Diagram of experimental setup](image)

**Figure 1.** Schematic diagram of the geometrical arrangement of the experiment.

For the experiments (figure 1) the spot size at the focus of the beam was 50 µm in diameter. The laser beam was incident to the target under an angle of 45° with respect to the target normal. The organic samples (tooth and bone) were cut by a diamond saw into 1mm thick slices and were mounted on a xyz rotating target holder. The experiments were performed with a mode-locked Ti:sapphire oscillator-amplifier laser system and an ArF excimer laser. The femtosecond system is utilizing a chirped pulse amplification technique, which has been described in detail elsewhere [15]. Briefly, the low energy seed pulses established in the oscillator are stretched before entering the amplifier and amplified in a multipass amplifier, after this the pulses are recompressed by sending them to a prism compressor. The output power of this system is in the order of 10¹⁵ W/cm² at 1 kHz repetition rate. The pulse duration is ≈ 25 fs. The ions signal is detected by a micro-channel-plate assembly (MCP) and then sent to a LeCroy transient recorder whose output is transferred through a GPIB interface to a computer for storage. In all cases every acquisition is carried out on a not previously irradiated surface. The ablation rates were measured over the intensity range 10¹³-10¹⁴ W/cm² with 30 fs, \( \lambda = 800 \text{nm} \) and at \( \lambda = 193 \text{nm} \) over the intensity range 10⁸-10⁹ W/cm², 30 ns.

4. Results and discussion

An important parameter in laser ablation is the ablation threshold, which can be formulated as the minimum applied energy fluence required initiating the removal of the material. The ablation threshold varies from material to material depending on the material structure and properties, such as the ionization potential, as well as on the laser pulse duration and wavelength. By varying the laser parameters in a controlled way, very precise examination of the ablation process can be performed at various wavelengths. As already reported [14,15] the femtosecond laser ablation mass spectra of tooth and bone samples shows the presence of many peaks attributable to the presence of organic \( C_mH_n \) ions, fragment ions with masses up to \( m/z=290 \) and inorganic masses (figure 2) like \( \text{Ca}^{2+} \) etc.

In the current experiment the ion yield for the most prominent species is obtained by integrating the main peaks for different laser energies.
Figure 2. TOF mass spectra of ablated species of tooth sample irradiated with 30 fs pulses, at $\lambda = 800\text{nm}$, intensity $I = 5.1 \times 10^{13} \text{W/cm}^2$ (a) and with 30 ns pulses, at $\lambda = 193 \text{nm}$, intensity $I = 2.5 \times 10^8 \text{W/cm}^2$ (b).

Figures 3 and 4 present the ablation signals from bone sample acquired with integration over one peak as a function of the laser intensity for low mass fragments. It is observed that the intensity dependence is the same for each individual peak. At low laser power densities the signals increase slowly and irregularly. The signals increase drastically when power density above $3 \times 10^{14} \text{W/cm}^2$ is reached. At higher laser power densities the signal intensity reaches a maximum. The most efficient ablation (optimal signal detection without the onset of considerable fragmentation) was observed in the intensity range between $3.5 \times 10^{14} - 4 \times 10^{14} \text{W/cm}^2$. Optimum laser intensity is desirable for ablation of intact molecules with minimal internal excitation that may lead to dissociation, prior to direct ionization. In order to have intact ionization, two dissociation mechanisms have to be circumvented, one is the absorption-dissociation-ionization (ADI), and the other one is absorption-ionization-dissociation (AID) mechanism. The ADI process can be overcome by increasing the laser intensity, and intact ionization develops on a time scale that is faster than the neutral dissociation time scale. When the laser pulse length is shorter than the lifetime of the dissociative state the up-pumping rate may become so high that the ionization level is reached. The rapid ionization will avoid intramolecular energy transfer, which can lead to excitation or fragmentation of intermediate states. In the case of femtosecond excitation, the ADI is suppressed and the ladder climbing mechanism is expected to be dominant as the pulse width decreases.

We observed a lowering of the ablation threshold in comparison to nanosecond ablation. This observations lead to the conclusion that the process of molecular fragmentation develops very fast in

![Figure 3](image1.png)  
**Figure 3.** Intensity of Na ion peak signal of bone sample, $\lambda = 800 \text{nm}$, 30 fs.  

![Figure 4](image2.png)  
**Figure 4.** Intensity of K ion peak signal of bone sample $\lambda = 800 \text{nm}$, 30 fs.
ultra-short laser ablation and is in good agreement with the theoretical predictions [16]. For large molecules the mechanism of ionization has been discussed by De Witt and co-workers. They observed less fragmentation of molecules in the lower intensity regime of $4 \times 10^{13}$ W/cm$^2$. Comparative studies were carried out on tooth samples under ns irradiation over a range of laser intensities. Figure 5 compares the response of the three most intense masses under irradiation at 193 nm.

It was found that for 30 ns pulses the ablation threshold for the tooth sample is in the order of $\approx 1.2 \times 10^8$ W/cm$^2$. As can be seen on figure 5, increasing the laser intensity lead to an overall increase of the ion intensity signal (observed for $I > 1.5 \times 10^8$ W/cm$^2$). Furthermore, in figure 5 (as in figure 4) a sharp increase is well observed in the K ion peak intensity signal. In contrast to figure 3, where the signal intensity of Na ion peak increases abruptly at $I > 3.4 \times 10^{14}$ W/cm$^2$, the behavior in the ns range shows no sharp increase of Na and P signal over the whole range of intensities. There appears to be a uniform yield of Na and P ions at all laser intensities. Comparing the experimental results obtained for femtosecond and nanosecond intensity dependence, one can conclude that there is a great difference in the effectiveness of producing of ionized species of organic samples. This characteristic is probably caused by differences in the ablation mechanisms in ns and fs ablation regime.

![Figure 5. Dependence of peak intensity of laser ablated Na, K, and P ions of tooth sample vs. laser intensity at $\lambda = 193$ nm, pulse duration 30 ns.](image)

**Conclusion**

We have shown that the laser ablation induced by 25 fs and 30 ns laser pulses yields very interesting results in what concerns the production of ionized atoms species. Moreover, the mass spectrometric data suggest that the fs regime is characterized by detection of higher number of masses with respect to the ns regime. By tailoring the laser intensity we can obtain a gentle ablation of tissue material and achieve different modifications of the processed areas. The femtosecond ablation threshold was determined based on TOF-MS measurements. The analysis of the mass spectrometric data reveals the influence of laser intensity on the mass distribution. The experimental results show that the formation of ionized species is in relation with the laser fluence applied in the fs case. This characteristic gives a possibility for more efficient energy deposition leading to ablation with suppressed fragmentation of molecules, which is very valuable for a variety of medical applications and development of future therapies. However some irregularities present in the results prompt questions as to the accuracy and repeatability of the TOF-MS method for characterizing the ultra-short laser ablation mechanism of biological tissues, thus creating a need to extend the measurements by varying the pulse duration.

**References**

[1] Holzer M, Rabsilber M and Auffarth G 2006 Femtosecond laser-assisted corneal flap cuts: morphology, accuracy, and histopathology *IOVS* 47 2828-2831

[2] Deppe H and Horch H 2007 Laser applications in oral surgery and implant dentistry *Lasers Med. Sci.* 22 217-221

[3] Yip H K and Samaranayake L P 1998 Caries removal techniques and instrumentation: a review *Clin. Oral Invest.* 2 148-54
[4] Attrill D C, Farrar S R, King T A, Dickinson M R, Davies R M and Blinkhorn A S 2000 Er:YAG laser etching of dental enamel as an alternative to acid etching Lasers Med. Sci. 15 154-61
[5] Fried D and Breunig T S 2001 Infrared spectroscopy of laser irradiated dental hard tissues using the advanced light source Proc. SPIE 4249 99-104
[6] Rode V, Gamaly E G, Luther-Davies B, Taylor B T, Graessel M, Dawes J M, Chan A, Lowe R M and Hannaford P 2003 Precision ablation of dental enamel using a subpicosecond pulsed laser Australian Dental J. 48 233-9
[7] Ebihara A, Majaron B, Liaw L-H L, Krasieva T B and Wilder-Smith P 2002 Er:YAG laser modification of root canal dentine: influence of pulse duration, repetitive irradiation and water spray Lasers Med. Sci. 17 198–207
[8] Weigl P, Kasenbacher A and Werelius K 2004 Dental applications Topics Appl. Phys. (Springer) 167-87
[9] Minet O, Doerrschel K and Mueller G Lasers in biology and medicine Landolt-Boernstein New Series (Springer) 279-310
[10] Krueger J and Kautek W 2004 Ultrashort pulse laser interaction with dielectrics and polymers Adv. Polym. Sci. 168 247-89
[11] Kautek W and Krueger J 1996 Laser ablation of dielectrics with pulse duration between 20 fs and 3 ps Appl. Phys. Lett. 69 3146-48
[12] Henyk M, Mitzer R, Wolfframm D and Reif J 2000 Laser induced ion emission from dielectrics Appl. Surf. Sci. 154 249-55
[13] Chichkov B N, Momma C, Nolte S, Alvensleben F and Tünnerman A 1996 Femtosecond, picosecond and nanosecond laser ablation of solids Appl. Phys. A 109-15
[14] Daskalova A and Husinsky W 2004 Ultra-short laser ablation of biological tissue Proc. SPIE 5830 473-8
[15] Daskalova A and Husinsky W 2006 Detection of biological molecules from hard biological tissue using ion-beam-induced desorption and femtosecond laser ablation Plasma Process. Polym. 3 257-61
[16] Daskalova A and Husinsky W 2007 Ionization of biomolecules in high-intensity laser fields 2007 Proc. SPIE 6604 66042G1-5