Femtosecond lasers: powerful tools for biological hard tissue investigations

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Mass spectrometry can be used to identify the biomolecular species present in laser-ablated material from bone and teeth.

Ultrashort laser pulses represent ideal tools for the microstructuring of solid targets. Recently, they have also been used in a variety of new applications in nanosurgery and ophtalmology. For example, femtosecond-pulse medical implants can now be fabricated, such as the stents used to avoid bypass surgery when blood vessels are blocked by plaque.\(^1,2\) Figure 1 shows that these pulses can also be used to drill 20\(\mu\)m holes into vascular transplant grafts with high accuracy, less collateral damage and high reproducibility. In eye surgery, laser-assisted in situ keratomileusis (LASIK) procedures are now performed using ultrashort laser pulses that allow ablation of corneal tissue with high-precision tissue cuts and smaller local heating effects.

The use of femtosecond lasers is also gaining ground in dentistry. The introduction of lasers for the removal of hard dental tissue dates back to the mid-1990s. Initial efforts for their use in a clinical setting were focused on developing inexpensive hand-held devices with a high ablation speed. However, recent enamel and dentine ablation studies show that the use of conventional laser sources with nano- or microsecond pulse widths leads to cracking and uncontrolled material removal. The poor absorption of laser radiation by tooth material also requires high laser power, causing excessive heat deposition in the tooth being treated and thermal effects in the surrounding dental tissue. Recent reports however, show that no collateral thermal damage is observed with the use of subpicosecond laser pulses.\(^3\)

Ultrashort pulses provide efficient, fast and localized energy deposition, low deformation and ablation thresholds, as well as small molecular fragmentation levels. This, plus the high accuracy and reproducibility of femtosecond lasers has increased their potential as low-cost tools for a wide variety of surgical applications. Our current research aims to investigate laser ablation processes using biological samples such as tooth and bone as target materials. By studying the process of laser ablation, we seek to qualitatively improve the biomedical applications of femtosecond lasers.

Experimental set up

We examined the ablation process of biological samples by varying parameters such as pulse duration, laser wavelength, and laser fluence.\(^4\) In our approach, we combine ultrashort laser ablation to time-of-flight mass spectroscopy (TOF-MS). After removal of macroscopic amounts of matter from the surface of the material, TOF-MS is then used for the detection and trace analysis of the atoms and molecules ablated or sputtered from the samples. This material consists chiefly of polypeptides and proteins.\(^4\)

All experiments were carried out under ultrahigh vacuum (UHV) conditions.\(^5\) The UHV chamber was equipped with a...
Figure 2. Experimental femtosecond laser system consisting of a femtosecond Kerr-lens mode-locked mirror-dispersion-controlled Ti:sapphire oscillator pumped by a diode-pumped Verdi laser, a nine-pass chirped-pulse amplification Ti:sapphire amplifier (Omega Pro) pumped by a Nd:YLF laser. We used the femtosecond laser system shown in Figure 2 to deliver $\approx30\text{fs}$-pulses at a laser intensity of $10^{15}\text{W/cm}^2$ to ablate material from tooth and bones samples.

Mass spectrometry is a powerful tool for the analysis of molecular species on a solid surface. It has also proven most useful in physicochemical studies of biological laser-induced photo-decomposition products. Since we knew that the main components of our tooth and bone samples were collagen fibers and hydroxyapatite ($\text{Ca}_{10}(\text{PO}_4)_6(\text{OH})_2$), we expected to observe their dissociation products in the measured mass spectra of the ablated species.

Figure 3 shows the TOF-MS spectrum of the material ablated from a bone sample. Laser irradiation breaks the hydroxyapatite OH$^-$ bond, releasing H$^+$ ions and Ca ions. The spectrum shows a peak at $m/z=40$, attributed to Ca. The presence of residues from the collagen polypeptide chain is also confirmed by the reflectron TOF mass spectrometer. The mass spectrum of laser-ablated material from a bone sample irradiated with 30fs-pulses at $\lambda=800\text{nm}$ with a laser ablation intensity of $5.1 \times 10^{13}\text{W/cm}^2$ shows a peak at $m/z=40$ attributed to Ca. (See Figure 3, inset.)

Figure 4 shows the spectrum obtained for the material ablated from a tooth sample. As was the case for the bone mass spectrum, a Ca ion peak is also observed, but with much higher intense (see Figure 4, inset). Peaks assigned to $\text{C}_n\text{H}_m$ collagen fragments are also observed at $m/z=104, 113, 127, 130, \text{and } 155$. Continued on next page
Conclusion
Our work demonstrates the sensitivity of TOF-MS for the identification of the biomolecular species present in laser-ablated material from hard tissues. Performing femtosecond laser ablation at 800nm altered the chemical composition of the ablated tissue and yielded the highest number of characteristic ions. The results obtained on our tooth sample are especially interesting for dental surgery applications. They show that ultrashort pulses can be used as a minimally invasive technique to ablate dental hard tissue with minimal thermal damage to the surrounding tissues. Our preliminary results also confirm earlier reports showing that it is possible to process dental hard tissue with high efficiency and we are accordingly planning further reproducibility studies.3

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References