STUDY OF HARD AND SOFT BIOLOGICAL TISSUE COMPOSITIONS USING LASER-INDUCED BREAKDOWN SPECTROSCOPY

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Abstract - The biological tissue composition was studied in vitro during laser ablation, by using Fluorescence Spectroscopy of the ablation plume. The objective was to detect the elemental composition of the caries-free teeth, myocardium and chicken cartilage. The atomic elements that are responsible for the emission peaks present in the acquired spectrum were identified. Calcium (Ca) was the element that presented the larger number of emission lines in the studied wavelength range (425 to 610 nm). Calcium (Ca) and Magnesium (Mg) lines were found for all sample types. Phosphorus (P) could also be identified in caries-free tooth samples. Similarities and differences between hard and soft tissues could be identified in the luminescence spectra obtained from laser-generated plasma. The experimental results have shown the potential of LIBS for biological tissue discrimination.

Introduction

There is a need for discriminating differences between several types of biological tissues with reliability and minimal damage. Experiments employing nanosecond and picosecond laser for material removal has been reported [1-6]. Optical spectroscopy of the laser-removed material stands as a powerful tool to gather information on the physical phenomena involved in the ablation process. Laser ablation has been applied to the determination of elemental concentrations in a variety of materials such as metallic alloys [7-11], leaves [12], teeth [13-15] and biological organism [16-18], hair, nails and minerals such as apatite [19-22]. The detection of lead and other trace elements in biological samples is important in meat inspection, residue control, and standard reference material production, which can be used in a practical monitoring system for chemical contaminations. Laser-Induced Breakdown Spectroscopy (LIBS) can be used for real time discrimination of the type of tissue been hit by the laser [23-25].

The present work reports on the spectroscopic measurement of the plume generated by laser ablation of hard and soft biological samples such as dentin and dental enamel and myocardium tissue by using the Laser-Induced Breakdown Spectroscopy (LIBS).

Experimental Setup

A Q-switched Nd:YAG laser (1064 nm, 10 ns pulse duration) has been fired onto selected tissue targets at an incident angle of 90º from the surface. After focusing with a 40 cm focal length lens, the beam diameter at the sample surface was 0.8 mm, resulting in a laser fluence of 40 J/cm^2.

The plume luminescence was collected by a 600 µm fused silica fiber. The optical emission spectra have been acquired by means of a 0.25 m spectrograph furnished with a 300 lines/mm grating which covers the spectral region from 325 nm to 625 nm. An intensified CCD (Charge Coupled Device) with 256x1024 pixels was connected at the monochromator detector port. The specified CCD gating capability was 5 ns. The CCD gating and time delay was controlled by a delay generator. The wavelength calibration was performed by using a Hg-Cd-Zn calibration lamp and a red (632,8 nm), a yellow (594 nm) and a green (543,5 nm) He-Ne lasers. The collection fiber was positioned parallel to the sample surface and perpendicularly to the high energy laser beam, as in figure 1.

Freshly-extracted, caries-free human teeth, chicken myocardium and cartilage were used as study samples. LIBS measurements were performed in 30 enamel samples, 30 dentin samples, 60 chicken myocardium and 30 chicken cartilage samples. For the sake of data averaging, to improve signal to noise ratio, spectra were collected at several different points of the same sample or new sample pieces, were used.

The detector gate width could be measured and adjusted electronically to the desired time window. After adjusting electronically the desired acquisition time window, the laser was

fired three times and the accumulated spectra was acquired.

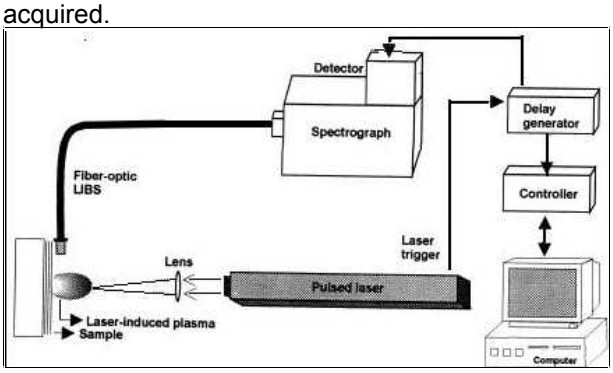

Figure 1: Schematic diagram of the experiment.

The sample was moved to a fresh point for a new acquisition. The data could than be evaluated for the repeatability and averaged for a better signal to noise ratio.

Results and Discussions

When the tissue is irradiated with a high energy laser pulse, the optical breakdown on sample surface can lead to plasma formation. Plasma fluorescence due to optical breakdown often precedes the bulk ejection of ablated material from the tissue or occurs simultaneously with ablation. In the later case, it cannot be separated from ablation product luminescence in a gaseous cloud or the particulate plume. Plasma optical breakdown is complete within a few picoseconds after the end of the incident laser pulse. Various spectra from laser-irradiated samples were obtained in order to identify the emission lines. Typical spectra collected during ablation of biological tissues are shown in Figure 2.

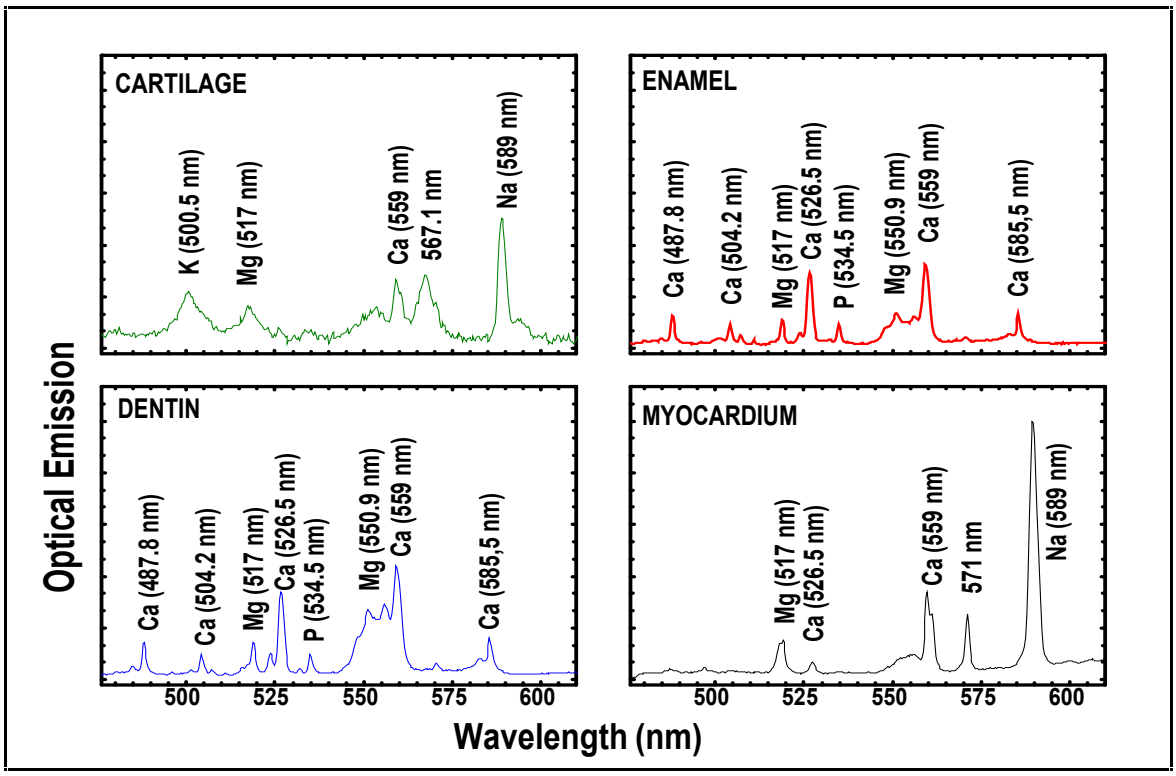

Figure 2: Typical spectra collected during ablation of biological tissues.

The spectral emissions from electronic transitions between atomic orbitals show up as thin lines, characteristic of each element. Many of the lines found in the probed spectral region for the different types of tissues studied herein exactly fit each other. The constitution of the observed spectra is complex: the lines are overlapping and may correspond to various atoms, molecules or radicals. Therefore precise identification of spectral lines is rather difficult. In this work, the Handbook of Chemistry and Physics [26] was used for the identification of the atomic emission lines.

Figure 3 highlights the spectral features common to two or more of the studied tissue types.

The majority of the observed emission lines corresponds to the Ca atom. Identified Ca lines are 487.8, 504.2, 526.5, 559 and 585.5 nm. The sodium doublet at 589.0 and 589.6 nm was identified by using a higher resolution grating (600 lines/mm). The calcium lines at 487.8 nm, 504.2,

526.5 nm and 585.5, as well as the P line at 534.5 nm were observed only in dentin and enamel. The Na doublet was observed as the most prominent line in spectra obtained for the cartilage and myocardium, this not being observed in dentin and enamel spectra. The lines at 500.5 nm and 567.4 nm were observed in no other tissue then cartilage, while the fluorine line at 570.4 nm was observed only for the myocardium.

The sodium emission doublet has been reported in the literature for different tissue types and others materials [27-29].

Figure 4 highlights the spectral features that are common to all the studied tissue types, i.e. the 517 nm and 559 nm lines, from Mg and Ca, respectively.

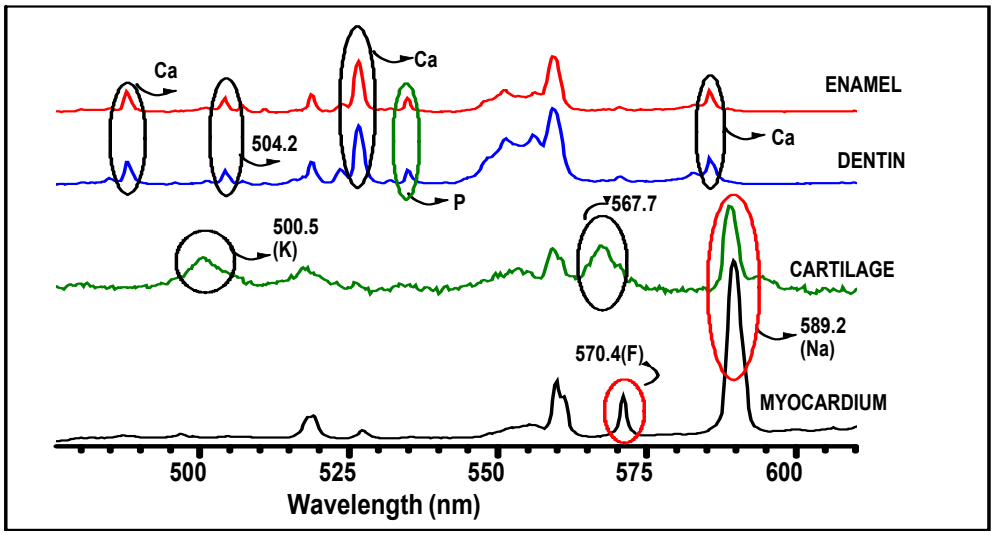

Figure 3: Characteristic LIBS spectra for soft (non-calcified) and hard (calcified) biological tissues.

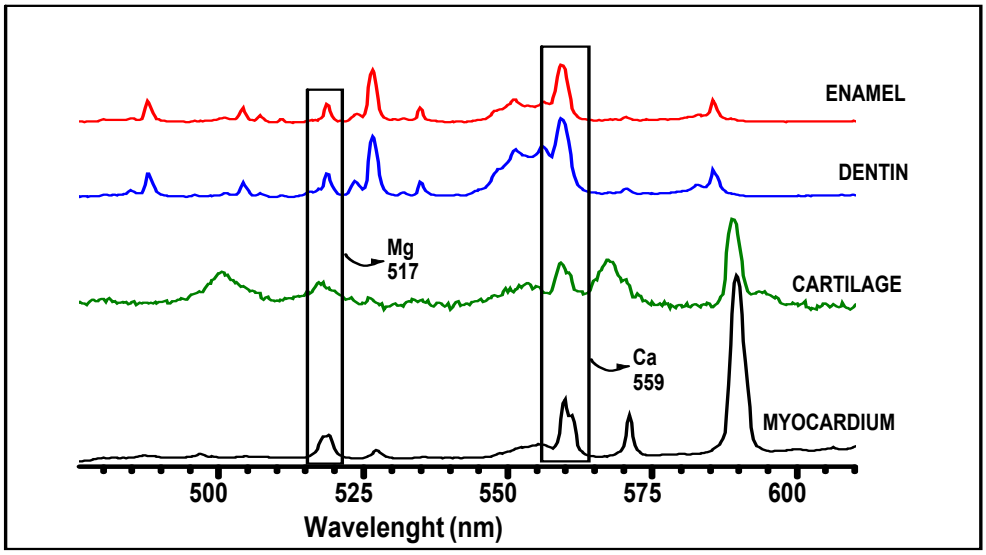

Figure 4: Spectral similarities between soft (non-calcified) and hard (calcified) biological tissues.

Conclusions

The experimental results have shown the capability of the technique known as LIBS for probing the biological tissue composition. In this work the LIBS revealed the presence of different atomic elements in the plasma produced in tissues of distinct natures.

The atomic elements that are responsible for the emission peaks present in the acquired spectrum were identified. Calcium (Ca) is the element that presents the larger number of emission lines in the studied wavelength range (425 to 610 nm). Calcium (Ca) and Magnesium (Mg) lines were found for all sample types. Phosphorus (P) could also be identified in caries-free tooth samples.

Similarities and differences between hard and soft

tissues could be identified in the luminescence spectra obtained from laser-generated plasma. The experimental results have shown the potential of LIBS for biological tissue discrimination.

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