STUDY OF HUMAN DENTARY TISSUES BY LASER-INDUCED BREAKDOWN SPECTROSCOPY

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Abstract - The aim of the present study was to evaluate the possibility of differentiating between non carious and carious dentin and healthy enamel from demineralized/ remineralized with fluor, using Laser-Induced Breakdown Spectroscopy (LIBS). A measurement system with an integrating sphere was used. A Q-switched Nd:YAG laser (1064nm, 10ns pulse duration) was used for sample ablation. The spatially integrated luminescence was collected by an optical fiber and sent to a spectrophotometer for spectral analysis. For this study, tooth samples were divided in four groups: A – non carious enamel, B - non carious dentin, and C - dentin in chronic carious process; D – demineralized/remineralized enamel with fluor. Spectral lines from specific atomic elements were identified, mainly Ca(362,6nm; 373,9nm; 393,4nm; 396,7nm and 585,5nm), Mg(517,4nm and 553,9nm), F(422,5nm and 470,4nm), Na(444,6nm), Mn(383,1nm), P(534,4nm), Si(500,6nm and 568,1nm) and Cl(372,6nm). The luminescence of the ablation products displays both mineralized and non-mineralized matrix composition of the dentin and enamel. Differences between carious, non carious dentin and enamel could be identified by LIBS.

Palavras-chave: LIBS, Teeth, Laser, Biomedical optics
Área do Conhecimento: III- ENGENHARIAS

Introduction

Human dentin, which forms the major bulk of the tooth structure, is described as a porous, partially fluid-filed, innervated mineralized tissue (MISERENDINO *et al.* 1995 [1]). Dentin caries involves the dissolution of mineralized matrix in an environment acidulated by bacterial activity. The dissolution of inorganic mineral contents in apatite like hydroxyapatite (HAP) has been studied. Variations in mineral composition can be qualitatively evaluated with Laser-Induced Breakdown Spectroscopy - LIBS (NIEMZ*,* 1996 [2]). Laser ablation of dentin yields a dense plume that can be ejected to a height of several millimeters above the surface with observed ejection velocity in excess of 1.200m/sec (PASHLEY,1992 [3]).

In this work, the laser-induced breakdown spectroscopy is applied for *in vitro* analysis of elemental composition of dentin and enamel samples. The LIBS analysis is potentially applicable during the laser drilling process of teeth (READER & CORLISS, 1990 [4]). The aim of the study was to evaluate the possibility of differentiating non-carious from carious dentin and demineralized/remineralized enamel with fluor using the LIBS technique.

Materials and Methods

Figure 1 shows the used LIBS system comprising the ablation laser, a converging lens for beam focusing, an integrating sphere with the sample holder placed in the sample port and an optical fiber at the sphere detector port for the collection of the plasma-emission. The optical signal collected by the fiber is coupled into a spectrograph for spectral analysis.

A Q-switched Nd:YAG laser (Quanta Ray, 1064nm, 10ns pulse duration) has been fired onto teeth sample targets at an incidence perpendicular to the surface. After focusing with a 40cm focal length lens, the beam diameter at the sample plane was 0,8mm, resulting in a laser fluence of 40J/cm^2.

The plume luminescence was collected by a 600µm fused silica fiber placed at the detector port of the integrating sphere. The optical emission spectra have been acquired with a 0,25mm spectrograph (Oriel Instruments MS257) furnished with a 300g/mm grating blazed at 400nm which covers the spectral region from 325nm to 625nm. An intensified CCD (Charge Coupled Device) with 256x1024 pixels was connected at the monochromator detector port. The specified CCD gating capability was 5ns. The CCD gating ant time delay was controlled by a model DG535 delay generator, from Stanford Research. The

spectrometer wavelength calibration was achieved by using spectral lines of a Hg-Cd-Zn lamp, a green He-Ne laser operating at 594nm and an additional 4mW laser at 532nm. The accuracy of the wavelength calibration is estimated to be 0,5nm. The fiber was positioned parallel to the sample surface and perpendicularly to the high-energy laser beam, as in figure 1.

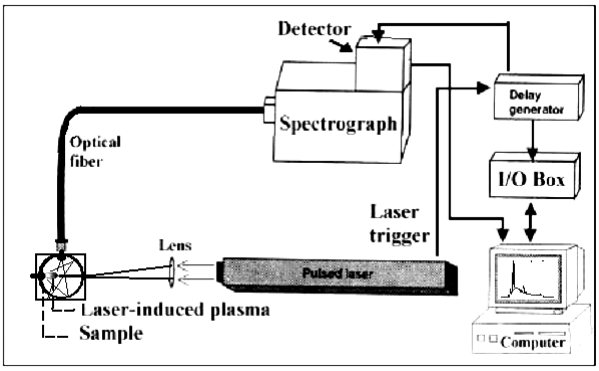

Figure 1: Schematic of experimental set-up.

Study samples.

Freshly extracted, carious and non carious permanent human teeth, obtained from a dental clinic with a protocol approved by a institutional review board, were used as study samples.

The teeth were cleaned using a water-pumice paste with a Robson brush mounted in a low-speed hand piece. The specimens were sectioned using a carbide bur mounted on a high-speed hand piece, refrigerated with air-water spray in $3mm^2$.

The samples were stored in distilled water until just before the experiment to avoid dryness. Thereafter, the teeth specimens were randomly divided in four groups (n=4) as described below and in the table 1. The fourth group suffered a pH cycling, which was made in 9 cycles with the demineralizing solution at last of 6 hours and the remineralizing at last of 18 hours.

Table 1: Experimental groups

Groups	Number of Samples
1 – non-carious enamel	n = 15
2 – non-carious dentin	n = 30
3 – carious dentin	n = 30
4 – demin./remin. enamel with fluor	n = 30
Total of samples	**n = 105**

The system for spectral analysis

An integrating sphere prototype was used for plume luminescence detection. The sample were positioned into an appropriate support into the integrated sphere as in figure 2.

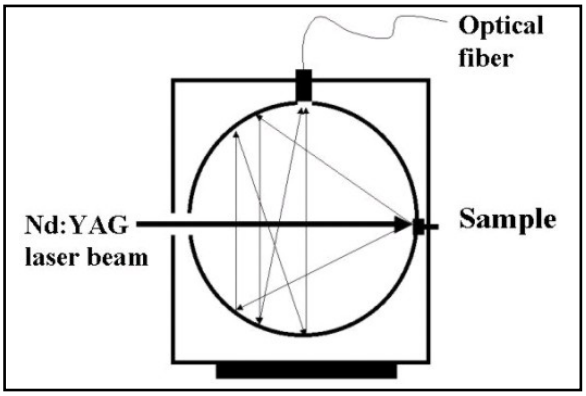

Figure 2: Arrangement for the measurements with integrating sphere.

To improve signal to noise ratio several spectra were collected and averaged for each sample each spectra being the cumulative signal obtained by firing the laser three times.

Results and Discussion

For laser drilling, the LIBS technique itself may be used for monitoring of the ablation process. The plasma, which is created during the ablation process, was spectrally analyzed. Using a fiber optic system, one could deliver the laser pulses to, and simultaneously collect plasma emission.

Changes in the relative composition would be indicative of different types of dentin: carious or non-carious and demineralized/remineralized enamel with fluor. The major constituent of the tooth's crystalline enamel and dentin structure is hydroxyapatite, $Ca_{10}(PO_4)_6(OH)_2$. Figure 3 shows strong emission lines from the plasma generated by laser ablation of dental tissues. The most prominent lines at 393,4nm and 396,7nm, occurs from the element Ca present in the mineralized dental matrix. For spectral lines identification we used the data tabulated by Reader & Corliss in the Handbook of Chemistry and Physics (SAMEK *et al*. 2001 [5]) Prominent O and H lines were not observed in the studied spectral range.

Selected LIBS spectra from dentin samples affected or not by caries and dental enamel are shown in figure 3.

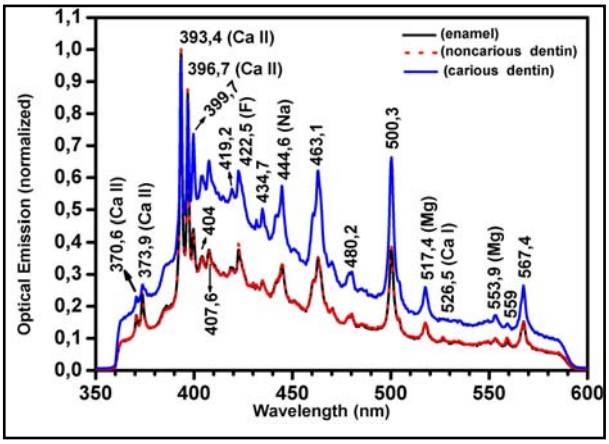

Figure 3: LIBS spectra for the experimental groups.

Typical LIBS signal obtained with the sample within the integrating sphere shows a structured spectrum superimposed to a broad background which is expected to carry contributions from the fluorescence of the dental matrix and from the ejected plasma.

Dental tissue fluorescence has been used as the basis of diagnostic methods for caries detection. The study of the broad emission observed in LIBS signals may be of relevance for caries research. Demineralization of dental enamel results in a loss of auto fluorescence.

Figure 4 shows the structured spectra for the healthy enamel experimental group. It can be observed that for healthy enamel the relative intensity of the 393,2 and 397,3 nm lines are diminished as compared to the case for demin./remin. enamel with fluor. Besides, in the healthy enamel was detected a wide range of elements: Na, N, Ca, F and Mg.

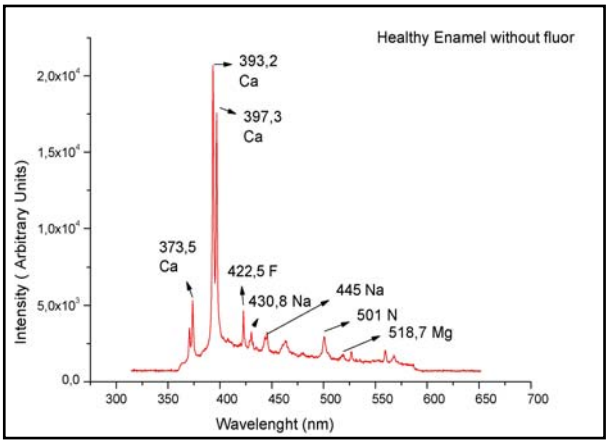

Figure 4: LIBS spectra for Healthy Enamel without fluor (average of 30 samples).

Figure 5 shows the structured spectra for the demin./remin. enamel experimental group with fluor in this case were detected the following elements: Ca, F, Zn, Si I, Si II, Cl e P.

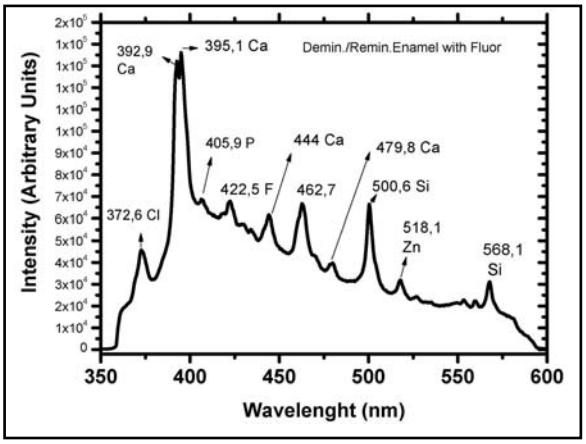

Figure 5: LIBS spectra for demineralized /remineralized enamel with fluor (average of 30 samples).

The spectral features of dentin, enamel and enamel with fluor spectra signals are very similar, but the found elements are different. For example, the absence of P, Cl and Zn in the healthy enamel.

For clinical applications, additional methodological aspects need to be explored. One caries detection system based on this principle which can be used in the general dental clinic or in clinical trials. For the elemental composition of extracted teeth, the LIBS method offers the possibility of absolute quantification, when properly calibrated. In the tooth samples that were analyzed we detected a wide range of elements, including Ca, F, Mg, Na, Sr and others.

For monitoring the ablation process of carious dentin in vivo during the drilling process, the ratios of matrix versus nonmatrix elements should be monitored, as suggested by Niemz (SERRA *et al*. 1998b [6])

Conclusions

The experimental results of the present study have shown the potential use of the LIBS technique for discriminating between noncarious and carious dentin, demin./remin. enamel with fluor and healthy enamel.

In this work, laser-induced breakdown spectroscopy is used to probe the presence of several atomic elements in the plasma generated by laser ablation of dental tissues. The plasma luminescence displays the composition of both, mineralized and non mineralized matrix of dentin. Differences between carious, non-carious dentin, demin./remin. enamel with fluor and healthy enamel could be identified by LIBS.

Exploiting the changes in concentration ratios between the matrix elements (Ca and P) and

nonmatrix elements (Mg and Zn), represented by relative changes in the line intensities seen in the LIBS spectra, discrimination of different tissue types or conditions could be achieved. Further developments could introduce LIBS in clinical practice, allowing the dentist to monitor and control the ablation process during the laser drilling of tooth.

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