Laser-Induced Breakdown Spectroscopy of Pathological Mineralized Dental Tissues in a Population of Senegalese Individuals: A Study of 75 Cases

Bintou C. Gassama1*, Moctar Gueye2, Alassane Traore3, Babacar Tamba1, Kawtar Babamou4, Soukeye D. Tine1, Ahmadou Wague3, Gerard Rey5 and Babacar Faye6

1Department of Oral Surgery, Department of Odontology, Faculty of Medicine-Cheikh Anta Diop University of Dakar, Dakar, Senegal
2Department of Prosthodontics, Department of Odontology, Faculty of Medicine-Cheikh Anta Diop University of Dakar, Dakar, Senegal
3Institute of Applied Nuclear Technology, Cheikh Anta Diop University, Dakar, Senegal
4General Practitioner, Casablanca, Morocco
5Paris Diderot University, Paris, France
6Department of Conservative Dentistry and Endodontics, Cheikh Anta Diop University of Dakar, Dakar, Senegal

Abstract

Attack by bacteria can induce structural changes in teeth. The aim of this study was to assess the spectra and the densities of mineralized dental tissue chemical components during cariogenic and periodontal pathologies in a population of Senegalese individuals.

Studies using in vitro laser spectroscopy or laser induced breakdown spectroscopy (LIBS) of teeth extracted from selected patients in a hospital have allowed various chemical constituents to be observed. Alterations in these constituents provide an indication of physiological changes in the hard dental tissues which could be affected by caries or by periodontal disease. Hence, LIBS represents a powerful tool to detect dental constituents and for the prevention and biological monitoring.

Keywords: Tooth; Chemical Component; Caries; Periodontal Disease; Laser Induced Breakdown Spectroscopy

Introduction

Dental caries and periodontal diseases are the main oral health problems that are encountered, and they are the principal reliable indicators of oral health [1]. The prevalence of these pathologies is steadily increasing in Africa and in rest of the world, with a wide geographical distribution and a progressive degree of severity. Aside from trauma, they constitute the main reason for tooth loss, thereby impacting on the quality of life and more broadly on the economy [2,3]. Indeed, in Africa, dental caries and their accepted complications presented 68.7% of the reasons for extractions undertaken by dental practitioners [4]. Furthermore, these prevailing pathologies particularly affected the ultrastructure of the targeted teeth. Human teeth are considered to be entities that have potential as individual and communal bioindicators. Their study has allowed for information to be gained retrospectively regarding nutrition, physiological stress, and exposure to environmental pollutants [5-7]. Bacterial attack, implicated in the appearance of cavities and periodontal lesions can induce changes in the chemical composition of teeth. These structural alterations over the course of the pathological process have been the subject of numerous studies [8]. Arshed, et al. [9], have shown that the elements found in all tooth samples are fluorine (F), phosphate (P), sulfur (S), chlorine (Cl), calcium (Ca), vanadium (V), chromium (Cr), magnesium (Mg), manganese (Mn), iron (Fe), cobalt (Co), and zinc (Zn). Other components such as nickel (Ni), copper (Cu), titanium (Ti), selenium (Se), and potassium (K) were only detected occasionally, while low levels of fluorine (F) have been shown to occur in teeth affected by caries or periodontal disease [10].

Analysis of calcified dental tissues by laser-induced plasma or laser-induced breakdown spectroscopy (LIPS or LIBS) allows for early differentiation of carious-bearing versus healthy tissues. It also permits the monitoring of the accumulation of trace of metals in teeth [11,12], which gives an indication of environmental exposure.

The aim of this study was to assess the spectra analysis and the density of chemical constituents of mineralized dental tissues based on cavity-forming and periodontal pathologies in a Senegalese population.

Materials and Methods

Adult patients from the oral surgery wards of several hospital centers in Dakar were targeted for the experimental study. Melanoderma patients who exhibited caries, periodontal lesions, or ectopias, between 18 and 86 years old were included in this selection. Such tooth collections were performed using extraction from the upper and the lower jaw. The retained teeth were healthy and assigned as reference compared with affected teeth. Patients with temporary teeth or fully decayed crowns were not included in the study.

The collected teeth were disinfected with sodium hypochlorite (5% free chlorine) and then soaked in physiological serum. Each tooth was stored in a labeled jar with the necessary information for identification of the patient. A cross-sectional cut using a hand-held bur allowed the enamel, the dentin, and the cementum to be exposed. Analysis of chemical constituents by laser spectroscopy was performed at the Institute of Applied Nuclear Technology (IANT) of the Cheikh Anta Diop University in Dakar.

A Q-switched Nd: YAG laser, 1064 nm, 200 mJ LIBS system was used (Ocean Optics) (Figure 1). A single nanosecond laser pulse N = 10 was sufficient to excite the dental sample.

The technical approach was based on laser induced breakdown spectroscopy analysis. Firing the laser shot on the mineralized tissues was performed on the surface of the tooth samples with a 60-75mm focal-distance lens producing a spot of about 250μm. The ablation of the small materials from the surface yields to the formation of the plasma. This dense matter is about a mixture of ions, atoms, electrons and molecules. LIBS spectra were measured in ambient air. This emission from the surface through fiber optic is used (Ocean Optics) (Figure 1). A single nanosecond laser pulse N = 10 was sufficient to excite the dental sample.

The collected spectra will be used to determine the density of each element in the samples analyzed (Figure 1).
Statistical analysis was performed using SPSS (Statistical Package for the Social Sciences) and Origin software for the spectra. The data was processed with R software. A multivariate analysis allowed p-values to be obtained and correlation tests between the various parameters to be carried out.

Results

Seventy five patients were examined, in which 63% were women and the sex-ratio was 0.6. The most highly represented age group was between 20 and 30 years old, and the average age was about 40 years.

The main purpose for the most consultations was functional (93% of cases) (Table 1). Most of the dental extractions were done in presence of light for the patients having cavities, with a substantial predominance of mandibular molars. This was found to correlate with age (0.000) and gender (0.029).

The various minerals detected using LIBS were Ca, Na, K, C, F, N, Sn, Fe, Vn, Si, O, and Cl. For K, F, Na, N and C, the periodontal disease highlights an average values that was higher than those of teeth with cavities. Yet when the caries were associated with periodontal disease, lower values for F, N, Ca and K were observed. Cavity indicators for calcium (81.91) were lower compared to periodontal indicators (102.5) (Table 2).

Ca, N and Na were presented in more quantities in teeth with cavities or periodontal disease. Conversely, lower spectral densities of potassium were seen when the two pathologies were associated (Figure 2).

For dentin, the K, N, and Ca constituents were decreased when the tooth was affected by a cavity. Higher densities of potassium and nitrogen were detected by LIBS when the cavities were associated with periodontal disease (Figure 3).

For the cementum, calcium and sodium had higher spectral densities when the tooth was affected by a cavity and when the two pathologies were associated (Figure 4).

Discussion

Mineralized dental tissues are biological samples that can hold considerable information. Elemental analysis by laser-induced breakdown spectroscopy (LIBS) of human teeth has been used to identify the chemical composition of teeth affected by carious lesion (CL), periodontal disease (PD) or carious lesion associated to periodontal disease (CLPD).

For this purpose 75 dental samples were extracted based on the presence of different pathologies mentioned above. Cavities justified the majority of the tooth extractions. In this study, 63% of extract teeth were affected by carious lesion (CL), 14.5% by periodontal disease (PD), 17.1% were affected by carious lesion associated to periodontal disease (CLPD) and the others by retention (Table 1).

For the cementum, calcium and sodium had higher spectral densities when the tooth was affected by a cavity and when the two pathologies were associated (Figure 4).

Table 1: Distribution of the samples according to clinical characteristics

<table>
<thead>
<tr>
<th>Clinical characteristics</th>
<th>Percentage (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Purpose for the consultation</td>
<td>93</td>
</tr>
<tr>
<td>Functional</td>
<td>7</td>
</tr>
<tr>
<td>Esthetics</td>
<td>14.5</td>
</tr>
<tr>
<td>Diagnosis</td>
<td>63</td>
</tr>
<tr>
<td>Periodontal disease</td>
<td>17.1</td>
</tr>
<tr>
<td>Cavity + periodontal disease</td>
<td>5.3</td>
</tr>
<tr>
<td>Cavity</td>
<td>86</td>
</tr>
<tr>
<td>Ectopic eruption</td>
<td>82</td>
</tr>
<tr>
<td>Type of extraction</td>
<td>86</td>
</tr>
<tr>
<td>Simple extraction</td>
<td>14</td>
</tr>
<tr>
<td>Surgical extraction</td>
<td>18</td>
</tr>
<tr>
<td>Site involved</td>
<td>82</td>
</tr>
<tr>
<td>Anterior – lateral</td>
<td>18</td>
</tr>
<tr>
<td>Posterior</td>
<td>0.029</td>
</tr>
</tbody>
</table>

For the cementum, calcium and sodium had higher spectral densities when the tooth was affected by a cavity and when the two pathologies were associated (Figure 4).
This pronounced morbidity of mandible molars can be explained by retention of soft deposits fermentable sugars which yield the demineralization of tooth tissues [4].

Analysis by LIBS of teeth affected by cavities or periodontal disease allowed detecting the chemical composition of dental tissues. Variations of components provide an indication regarding physiological changes in hard dental tissues that are affected by cavities of periodontal disease.

In our LIBS study we found that the calcium was the most common chemical element in the teeth. In addition it is one of the major minerals in the teeth mineralized tissues [13]. In figure 1C, 2 and 3 one can see that the loss of calcium content was more pronounced in teeth with cavities. Other studies have obtained the same result [14,15] showing that the decrease in the calcium content in enamel during a carious lesion is linked to the replacement of calcium in the Hydroxyapatite by other minerals. Furthermore, these authors concluded that LIBS is a potentially useful in vivo/in vitro tool for real-time identification of caries of drilling or cleaning of a cavity [16,17].

Other constituents such as O, K, F, Na and C were also detected (Figures 1C, 2 and 3). Higher average amounts of these constituents...
Figure 3: Variation in the chemical composition of dentin tissues (K, N, Na, and Ca) according to the pathologies. CL: CLPD: PD

Figure 4: Variation in the chemical composition of cementum tissues (K, N, Na, and Ca) according to the pathologies. CL: CLPD: PD
were seen with periodontal teeth compared to those with cavities. This indicates that the chemical changes in mineralized tissues are more pronounced when the cavity is associated with periodontal diseases. Lower amounts of K, F, Na, and N detected in the teeth enamel with cavities, this can be explained by the presence of a multi-factorial and polymorphic attack during dental pathologies. Based on the result that we have obtained we are able to provide evidence for higher concentrations of Ca and K in the enamel, as compared to the dentin. LIBS allows in vitro detection of dental chemical components, which variations are important factors for the identification of affected tissues. Studies showed that the content of calcium and phosphorous decreased while going from the enamel to the cementum [18,19]. In this study one can see also that over the course of periodontal disease, there were variations in the concentrations of calcium intensities. We think that these variations could be linked to the degree of periodontal attack of the cementum by bacteria as shown Aleo’s study quoted by Grotra, et al [19]. The latter observed hyper-calciification or a decalcification linked to the presence of bacterial endotoxins absorbed on the surface of the cementum.

Regarding the fluorine contents, the changes in the amounts detected were less pronounced in affected teeth by the different diseases. From Table 2, there are indications showing the decrease of fluorine content in CL and CLPD compare to healthy retention teeth. This could be explained by the fact that fluorine can form a bond with the Hydroxyapatite matrix to form fluorapatite, which increases the resistance of teeth to attack. In their work Arshed, et al [9], observed an unequal distribution of fluorine contents over time showing that, the concentration of fluorine decreased upon everyday use, and as a result of abrasive events; whereas it is increased by nutritional and water intake as well as through use of various fluorinated products [9].

In this study, as one can see to the table 2, presence of iron, vanadium, tin, and silicon were detected only with carried teeth; these quantities were too low to account for significant accumulation of toxic metals. However, their presence may be linked with cutting tools (bores) used during the handling of dental sample preparation.

Furthermore, analysis of calcified dental tissues by LIBS has allowed the accumulation of trace metals in teeth to be monitored [10,11]. This hence represents an indicator of environmental exposure of the patients. It could be used to prevent the accidental exposure of populations to chemical pollutants in underdeveloping countries.

Mineralized dental tissues are hence reliable bio-indicators. Our LIBS studies have shown a decrease in the principal constituents (Ca, Na, K, F, O, Cl) of teeth with carious lesion as compared to teeth in cases of periodontal disease. The structural changes, linked primarily to chemical bonds in the mineralized dental tissues, constitute an important means for monitoring the dental tissues over the course of pathological developments.

**Conclusion**

Investigation of hard dental tissues by laser induced breakdown spectroscopy (LIBS) has shown that there is a high level of variability in their chemical composition over the course of the development of carious lesion (CL), periodontal disease (PD) and combined CL-PD. The changes in terms of chemical composition detected by LIBS allowed us to distinguish the specificity of dental area. This study was performed in order to clearly identify the affected tissues based on a decrease in the concentrations of chemical components. Hence, LIBS constitutes a powerful tool to detect the basic constituents, in teeth affected, and for biological monitoring. Through such studies, it is possible to establish a link between entities detected in toothpastes, dental amalgams, and other restorative components with those present on the teeth, as well as to monitor the accumulation of toxic metals due to exposure to these dental materials [20].

For future studies, spectro-chemical analysis of teeth or other calcified tissues could also be of relevance to areas relating to legal-medical science, as well as for oral surgery following exercises of calculus during salivary lithiasis.

**Acknowledgment**

The authors would like to thank the manufacturers for supplying materials for this study.

**References**


