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ORIGINAL ARTICLE

Laser-induced breakdown spectroscopy analysis of human deciduous teeth samples

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Abstract Laser-induced breakdown spectroscopy (LIBS) analysis of human deciduous teeth has been performed by employing Nd:YAG laser (1064 nm, 10 ns) for the evaluation of plasma parameters as well as elemental analysis. The plasma parameters, i.e., electron temperature and electron number density of laser-induced teeth plasma at various fluencies, have been evaluated. Both parameters show an increasing trend up to a certain value of laser fluence, i.e., 2.6 J/cm^2. With further increase in laser fluence up to a value of 3.9 J/cm^2, a decreasing trend is observed which is due to shielding effect. With further increase in laser fluence up to a maximum value of 10.5 J/cm^2, the insignificant changes in plasma parameters are observed which are attributed to saturation phenomenon governed by self-regulating regime. Emission spectroscopy results exhibit that laser fluence is the controlling factor for both plasma parameters. The elemental analysis was also performed at constant laser fluence of 2.6 J/cm^2 by evaluating the variation in detected elemental concentration of Ca, Fe, Sr, Zn, and Pb in three different parts of human teeth, i.e., enamel, dentine, and cementum. The lower concentration of Ca as compared to the standard values of CaCO$_3$ (self-fabricated pellet) reveals that enamel is the most deciduous part of the human teeth. However, at the same time, it is also observed that the highest concentration of micro minerals is also found in enamel, then in dentine, and lowest in cementum. Carious or unhealthy tooth is identified by enhanced concentration of micro minerals (Pb, Sr, Zn, and Fe). The highest concentration of micro minerals as compared to other parts of teeth (dentine and root cementum) and lower concentration of Ca as compared to standard CaCO$_3$ pellet in enamel confirm that enamel is the most deciduous part of the teeth.

Keywords Enamel · Dentine · Cementum · LIBS · Electron temperature · Electron density

Introduction

Laser-induced breakdown spectroscopy (LIBS) is one of the simplest techniques for the evaluation of plasma parameters as well as elemental concentration [1]. In this technique, an intense laser light is used to ablate the target material, and emission spectra from the plasma plume are probed with the help of a high-resolution spectrometer. The obtained spectra of the target material are useful for the determination of plasma parameters like electron temperature and number density and also for the elemental analysis [2].

The functioning of living tissues and organs depends upon the elemental composition and concentration [3]. The knowledge of elemental composition of an archeological artifact is very helpful for archaeologists, physicists, and anthropologists in characterization of various aspects of past populations. In this regard, the concentration of trace elements in calcified tissues gives information about the environment, diet, and living habits of human beings [1, 4].

The human tooth consists of three major parts—enamel, dentine, and root cementum. The entire root surface of the tooth is covered with an avascular mineralized tissue called root cementum. It forms an interface between root dentine and periodontal ligament due to its intermediary position [5, 6].

Hydroxyapatite is the main component of hard tissues of the human body including teeth. Enamel is the hardest calcified tissue of the human tooth composed by 96 wt% of inorganic

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material, mainly hydroxyapatite and 4 % of organic material. Dentine consists by weight of 70 % mineralized inorganic material, 20 % organic material, and 10 % water. Cementum is composed by weight from 65 % mineralized inorganic material, 23 % organic material, and 12 % water [7]. The tooth enamel is the most mineralized tissue of the human body. This inorganic material is mainly composed by a calcium phosphate related to the hexagonal hydroxyapatite [8].

The quantitative analysis of detected element accumulation in teeth using LIBS has been investigated by various research groups [1, 9, 10]. Samek et al. [11] have investigated the close link between the elements found in toothpastes and tooth filling with those present in the teeth of different age groups of children. Thareja et al. [12] have investigated the elemental composition of healthy and deciduous part of the human tooth using LIBS analysis. It is also reported that the combination of LIBS and discriminant analysis provides a useful tool for identification of caries in human teeth [13]. The electron temperature and number density of teeth and bone have been investigated by Samek et al. [14].

The aim of the present work is to evaluate the plasma parameters, i.e., electron temperature and electron number density of dentine portion at different laser fluences ranging from 1.3 J/cm^2 to 10.5 J/cm^2. The variation in plasma parameters with laser fluence is revealed. The purpose of the work is to optimize laser fluence to explore optimized plasma parameters suitable for contamination-free and uniform ablation. The plasma temperature and plasma density are the two most important parameters that are representative of plasma characteristics [15]. The maximum plasma temperature and electron density are representative for maximum ablation efficiency as well as laser energy deposition to the target material. The aims of the present investigations are to explore the variation in two plasma parameters at various fluences and to find out the self-regulating regime. Within this working regime of fluence, the insignificant changes in plasma parameters occur which are responsible for uniform and stable ablation of teeth, e.g., drilling of micro holes for cavity preparation and selective removal of caries by laser.

According to our best knowledge, no work is reported in which the effect of laser fluence on laser-induced plasma parameters of dentine has been investigated.

The second motivation behind this work is to investigate variations in concentration of detected elements in three different parts of the teeth, i.e., enamel, dentine, and cementum. The purpose of the work is to evaluate the reduced concentration of matrix element, i.e., Ca and enhanced concentration of non-matrix elements or micro minerals (Sr, Pb, Fe, and Zn) in the three parts of human teeth sample. A comparison of matrix element Ca is done with $CaCO_3$ (self-fabricated pellet) because it is reported in the literature that physical properties of $CaCO_3$ strongly resemble with those of hydroxyapatite ($Ca_{10}(PO_4)_6(OH)_2$) [14].

This data helps to identify the most deciduous part of the human teeth. This elemental analysis also provides information of which element is responsible for the decay of the teeth and is helpful for the accurate medication and treatment that could be provided to patients.

Experimental details

Four deciduous human teeth were obtained and preserved in saline solution. To evaluate the plasma parameters, two samples were prepared by cutting the teeth samples in horizontal direction in the form of slices with dimensions of 7 mm× 2 mm×2 mm. For the purpose of elemental analysis, two samples of teeth were prepared by cutting in vertical direction with dimension of 10 mm×5 mm×2 mm. These samples were grinded and polished. The polished samples were autoclaved and ultrasonically cleaned in distilled water for 30 min before exposure. In order to compare the concentration of matrix element Ca with the reference data, the $CaCO_3$ pellet with dimensions of 10 mm×2 mm was fabricated by using fine powder of $CaCO_3$.

Nd:YAG laser (CRF200: Big Sky Laser Technologies, Quantel, France) with the wavelength of 1064 nm, pulse duration of 10 ns, repetition rate of 10 Hz, and maximum pulse energy of 400 mJ was employed as the source of irradiation.

Targets were mounted in target holder and were placed inside a stainless steel vacuum chamber which was evacuated to a base pressure of 10^{-3} Torr. In order to enhance the emission intensity of laser-induced plasma, the chamber was filled with Ar gas at pressure of 20 Torr. After passing through a lens of focal length of 50 cm, the laser beam hit the tooth sample at angle of 90° with respect to its surface.

The following two sets of experiments were performed:

1. The first set of experiment was performed by exposing the targets (cut in horizontal direction) at eight various fluences—1.3, 2.6, 3.9, 5.3, 6.6, 7.9, 9.2, and 10.5 J/cm^2. The emission spectra generated by laser-induced tooth plasma were collected through optical fiber and was focused through a lens of focal length of 5 cm. It was then collected by LIBS2500-7 spectrometer system (Ocean Optics Inc, USA).
2. The second set of experiment was performed for elemental analysis. For this purpose, vertical tooth samples were selected and were exposed to laser beam. Three different parts of teeth sample, e.g., enamel, dentine, and cementum, were focused one by one by moving the target in z-direction with the help of micrometer. For this set of experiments, the fluence was kept constant for all exposures, i.e., 2.6 J/cm^2.

Results and discussion

Plasma parameters

Plasma parameters, e.g., electron temperature and electron number density, have been evaluated as a function of laser fluence. Figure 1a represents the laser-induced breakdown spectra of tooth plasma obtained at laser fluence of 2.6 J/cm^2 under ambient environment of argon at pressure of 20 Torr. The emission spectrum contains spectral lines that correspond to neutral state of Ca I and different micro minerals, i.e., Pb, Zn, Sr, Fe, and O in neutral as well as in singly and doubly ionized ionization states. The detected lines are at 558.626 nm (Zn II), 585.53 nm (O I), 588.794 nm (Sr III), 612.024 nm (Fe I), and 616.024 nm (Pb II) [16]. For evaluation of plasma parameters, emission lines of matrix element Ca are selected at 458.14, 458.58, and 610.272 nm (NIST database). The wavelength at 458.58 nm of Ca I in Fig. 1b is utilized for calculation of electron number density.

For the calculation of electron temperature, population of excited states of plasma follows the Boltzmann distribution governed by the following relation [17, 18]:

$$\ln(I_{mn}\lambda_{mn}/g_{mn}A_{mn}) = -(E_m/KT_e) + \ln(N(T)/U(T)) \quad (1)$$

where λ_{mn}, I_{mn}, g_m, and A_{mn} are the wavelength, intensity, statistical weight, and transition probability between the transition states of m (upper level) and n (lower level), respectively. $U(T)$, $N(T)$, K, E_m, and T_e are partition function, total number density, Boltzmann constant, energy of the upper level state, and electron temperature, respectively. For a given spectrum, a Boltzmann plot of the logarithmic term ($\lambda_{mn}I_{mn}/g_mA_{mn}$) versus E_m yields a straight line whose slope is equal to $-1/kT_e$ under the assumption that the distribution is Boltzmann. Table 1 represents the spectroscopic parameters for the selected transitions lines of the laser-induced teeth plasma taken from the NIST database [19]. The transition lines 458.14 ($^3D_2 \rightarrow {}^3F^0_3$), 458.58 ($^3D_3 \rightarrow {}^3F^0_4$), and 610.272 ($^3P^0_0 \rightarrow {}^3S_1$) are used for

the evaluation of electron temperature. The evaluated value of electron temperature ranges from 5189 to 8288 K, and its variation with laser fluence is shown in Fig. 2a. The electron temperature increases with increasing laser fluence from 1.3 to 2.6 J/cm^2. With the increase in laser fluence from 2.6 to 3.9 J/cm^2, electron temperature decreases. With the further increasing in laser fluence from 3.9 to 10.5 J/cm^2, a saturation region with insignificant changes in excitation temperature is achieved.

The electron number density (N_e) is evaluated from the measured Stark broadened line profile of an isolated line of either neutral atom or singly charge ion. The full width at half maximum (FWHM) of the Stark broadening profile is related with the number density through the following relation [17, 20]:

$$\Delta\lambda_{1/2} = 2\omega\left[N_e/10^{16}\right] \quad (2)$$

The Ca (I) line at 458.58 nm is used to calculate the electron number density.

The variation of electron number density with increasing laser fluence is shown in Fig. 2b. Its value at the lowest fluence comes out to be 1.5×10^{18} cm^{-3} at 1.3 J/cm^2 and attains its maximum value of 3.8×10^{18} cm^{-3} at laser fluence of 2.6 J/cm^2. With increase in laser fluence from 2.6 to 3.9 J/cm^2, electron number density decreases. With further increase in laser fluence from 3.9 to 10.5 J/cm^2, a saturation region is achieved with insignificant changes.

The trend observed in electron temperature and electron number density is divided into three regimes. In the First regime both plasma parameters increased and attain their maxima. With further increase of fluence, there is a sudden decrease in both plasma parameters. With the increase in fluences up to the maximum value of laser fluence, they get saturated in the third regime. This trend can be explained as follows: Initially, ablation rate of teeth sample increases with increase in laser fluence [21]. The ablated plume also interacts with the trailing edge of the incoming laser light and absorbs energy from it resulting in its further heating and ionization

Fig. 1 The emission spectrum of laser induced tooth plasma collected under ambient environment of Ar at 20 Torr (a) overall (b) for emission lines of Ca I
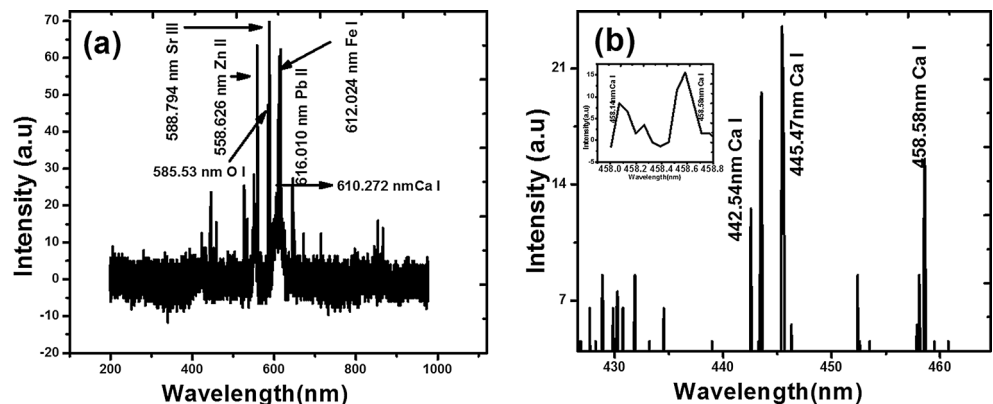
Table 1 The spectroscopic data for selected lines used to calculate the electron temperature and electron number density of laser induced tooth plasma [18]

Wavelengths (nm)	Transitions	Statistical weight g_m	Transition probability A (s^{-1})	Relative intensity (arbitrary unit)	Energy of lower level E (cm^{-1})	Energy of upper level E (cm^{-1})
458.14	$^3D_2 \rightarrow ^3F^0_3$	7	2.09e+07	23	20349.260	42170.558
458.58	$^3D_3 \rightarrow ^3F^0_4$	9	2.29e+07	24	20371.000	42171.026
610.272	$^3D^0_0 \rightarrow ^3S_1$	3	9.6e+06	27	15157.901	31539.459

[22]. This absorption occurs through mainly two processes, i.e., inverse bremsstrahlung (IB) and direct photoionization (PI), and results in generation of plasma with increased temperature and number density of charged particles [23]. The pressure at 20 Torr of Ar is also sufficient for confinement of ablated species, and more energy deposition from laser to generated plasma causes enhancement of T_e and N_e. After achieving a certain degree of ionization which corresponds to a critical density of electrons, plasma starts to shield the target from the laser beam [22]. This shielding effect is accounted for reduced ablation efficiency and consequently causes a decrease in the plasma parameters. The absorbed laser energy is now preferably converted to the kinetic energy of the species rather than for their ionization and heating. The plasma expansion and recombination losses result in the decrease of electron temperature and number density [24]. With the further increase in fluence, the saturation in these parameters is explained on the basis of formation of a self-regulating regime near the target surface. Plasma adjusts its density, temperature, and dimensions in such a way that it attains a constant amount of energy [25, 26].

Elemental analysis

The teeth samples utilized in our experiment were obtained from the dentist. These samples were diagnosed with caries lesions and extracted due to caries infection. The elemental analysis of three parts of human teeth, enamel, dentine, and cementum, is explored by LIBS analysis at a constant laser fluence of 2.6 J/cm², because at this fluence, maximum

intensity of trace minerals is achieved. Figure 1a represents the emission spectrum of various elements including of matrix element Ca as well as different micro minerals (Sr, Zn, Pb, and Fe). This spectrum was used to identify the elements and their relative concentrations. This spectrum exhibits that emission intensities corresponding to micro minerals Sr, Zn, Pb, and Fe are much higher than matrix element Ca. It reveals that the concentration of micro minerals is higher than that of Ca and represents the damage or caries in the teeth. This can be helpful for real-time detection of various elements and exact medication for dentists. The comparison of variation of the detected elements in three different parts of teeth, i.e., enamel, dentine, and cementum, is represented in Figs. 3 and 4. The variation in emission intensity of Ca I as a function of wavelength is exhibited for emission lines of 445.54 and 458.58 nm in Fig. 3a, b. The emission spectra of $CaCO_3$ pellet were also collected at laser fluence of 2.6 J/cm². This spectrum was selected as a reference for the comparison of Ca concentration. The variation in emission intensity represents the concentration of Ca in $CaCO_3$ as well as three parts of teeth sample. The highest concentration of Ca is detected in enamel and then in dentine and the lowest in cementum. Unnikrishna et al. [27] have observed the similar trend in the calcium concentration in these three parts of the deciduous tooth.

figure 4 shows the variation in emission intensity of detected micro minerals, i.e., (a) lead (Pb), (b) strontium (Sr), (c) zinc (Zn), and (d) iron (Fe) in the three parts of the deciduous human teeth. Again, a decreasing trend from enamel to cementum is observed for the detected micro minerals Pb, Sr,

Fig. 2 The variation of **a** electron temperature and **b** electron number density of laser-induced teeth plasma as a function of laser fluence
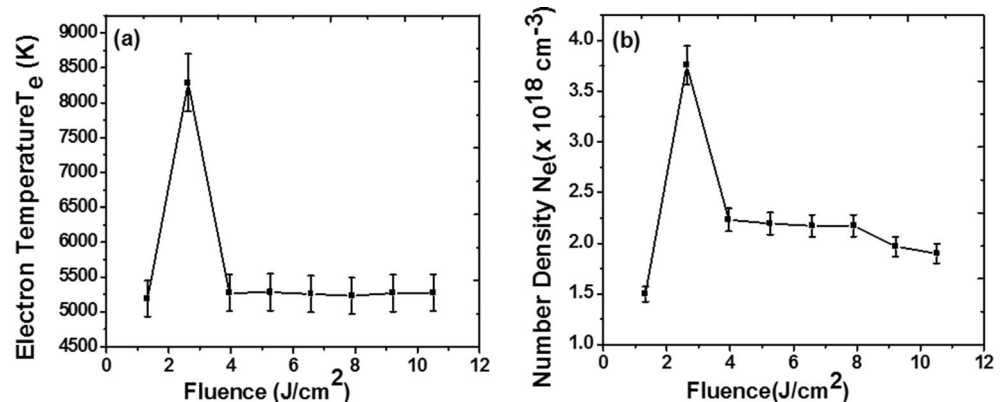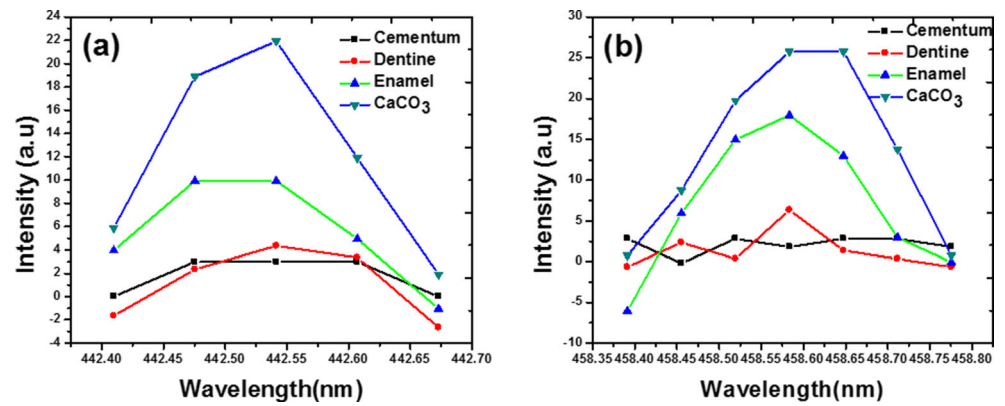
Fig. 3 The variation of Ca I in three different parts of deciduous teeth with respect to $CaCO_3$ for **a** 442.54 nm and **b** 458.58 nm


Zn, and Fe. Derise et al. [28] have reported that concentration of matrix element of Ca is higher in enamel than in dentine by using neutron activation and atomic absorption spectrophotometry methods.

Enamel is the most mineralized part of the human teeth. Carious part of human tooth can be identified through the decrease of matrix element Ca or increase in micro minerals [12]. The detected micro minerals are Fe, Pb, Sr, and Zn, where Pb is considered as a toxic element [14]. The highest concentration of micro minerals in the human teeth provides information of which part of the teeth is more damaged. Micro minerals are found in decreasing trend in the human teeth from enamel to cementum. The highest concentration of micro minerals in enamel indicates that the most deciduous part of human teeth is enamel [5]. The graphs of micro minerals in Fig. 4 represent the presence of detected micro minerals in enamel and also in other hard tissues of the human teeth, i.e., dentine and cementum. But, the highest concentration of micro minerals is detected in enamel, and it confirms the caries lesions or more damage of enamel as compared to the other tissues, i.e., dentine and cementum of the human teeth.

Fig. 4 The variation of micro minerals in the three different parts of the human deciduous teeth
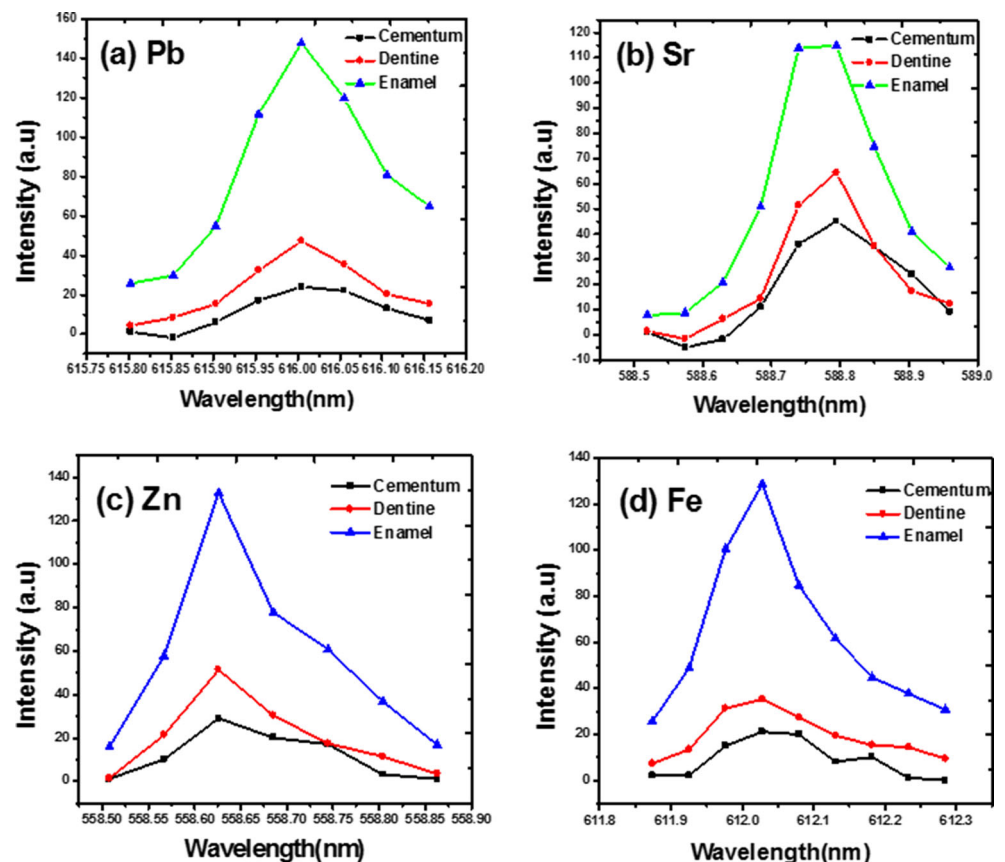
Conclusions

Laser-induced plasma parameters of teeth samples are evaluated at various fluences. The value of T_e ranges from 5189 to 8288 K where the value of N_e ranges from 1.5×10^{18} to 3.8×10^{18} cm^{-3}. Both plasma parameters increase with increasing laser fluence, attain their maxima at 2.6 J/cm^2, and then significantly decrease. After this decrease, these parameters do not change significantly, and a saturation behavior or self-regulating regime is achieved. These plasma parameters play a significant role for the ablation of dental samples. Therefore, the suitable selection of laser as well as plasma parameters provides the optimum conditions for required treatment of dental samples. The fluence corresponding to maximum electron temperature will be useful for maximum ablation efficiency and offer sterilization effects, by facilitating contamination-free condition during dental treatment. On the other hand, high fluences governing the self-regulating regime are more suitable for uniform and homogeneous dental ablation. The variation in concentration of different detected elements in deciduous teeth samples is also analyzed by LIBS analysis. The matrix element of calcium concentration is compared in three different parts of the teeth (enamel, dentine, cementum) with the artificially fabricated pellet of CaCO$_3$. Similarly, the concentration of Pb, Sr, Zn, and Fe in three parts of teeth are also compared. The highest concentration of trace elements is found in enamel, then in dentine, and lowest in cementum. This represents that enamel is the more affected area than all other parts of the teeth. Our purpose for applying the LIBS analysis for determining the elemental concentration is to identify the most affected area. The appropriate concentrations of various elements play a major role for identification of healthy and unhealthy teeth. This can solve health problems related to dentistry and can be helpful for proper and accurate medication.

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