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Mercury Amalgam Diffusion in Human Teeth Probed Using Femtosecond LIBS

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Abstract

In this work the diffusion of mercury and other elements from amalgam tooth restorations through the surrounding dental tissue (dentin) was evaluated using femtosecond laser-induced breakdown spectroscopy (fs-LIBS). To achieve this, seven deciduous and eight permanent extracted human molar teeth with occlusal amalgam restorations were half-sectioned and analyzed using pulses from a femtosecond laser. The measurements were performed from the amalgam restoration along the amalgam/dentin interface to the apical direction. It was possible to observe the presence of metallic elements (silver, mercury, copper and tin) emission lines, as well as dental constituent ones, providing fingerprints of each material and comparable data for checking the consistence of the results. It was also shown that the elements penetration depth values in each tooth are usually similar and consistent, for both deciduous and permanent teeth, indicating that all the metals diffuse into the dentin by the same mechanism. We propose that this diffusion mechanism is mainly through liquid dragging inside the dentin tubules. The mercury diffused further in permanent teeth than in deciduous teeth, probably due to the longer diffusion times due to the age of the restorations. It was possible to conclude that the proposed femtosecond-LIBS system can detect the presence of metals in the dental tissue, among the tooth constituent elements, and map the distribution of endogenous and exogenous chemical elements, with a spatial resolution that can be brought under 100 μm.

Keywords

Laser-induced breakdown spectroscopy, femtosecond LIBS, fs-LIBS, amalgam, dental restoration, mercury

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Introduction

Amalgam filling is a well established restorative material, mostly for posterior dental restoration, and has been widely used for many years in the twentieth century due to its mechanical and biochemical properties such as stability, low solubility, high compressive strength, pH and durability, besides the low cost, when compared to other restorative material such as composite resins. The amalgam is a metallic alloy generally formed by liquid mercury (Hg; 50%) and a matrix (50%), which is composed of silver (Ag; 45%), copper (Cu; 24%), tin (Sn; 31%), and sometimes zinc (Zn; 0–1%).[1] The mercury reacts chemically with the other metals, creating a malleable paste that quickly hardens and adapts to the dental cavity, facilitating the restoration of the damaged tooth. The main problem of amalgams is the presence of mercury, a metal known to be hazardous for the human health due to its biochemical reactions, the release of Hg vapors and Hg^{2+} incorporation in tissues,[2–4] which can cause harmful effects to various organs, neural and fetal development and problems, and to which no safe exposure levels have yet been established.[5,6]

In 2013, the Minamata Convention[7] of the United Nations Environment Programme (UNEP) agreed on the elimination of mercury from compounds for human use and, since long before this resolution, amalgam was being replaced by resin composites in dental restoration. This substitution is occurring in spite of the composites clinical disadvantages when compared to amalgam, which

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include reduced wear resistance,[8] increased microleakage,[9] marginal staining and the higher probability of secondary caries.[10] The resins clinical procedures are also more time-consuming and technique sensitive.[11] The amalgam advantages include decreased effect on subgingival microflora and bacterial biofilms,[12] easiness of removal that results in smaller enlargement of the original cavity,[13] being the least time-consuming to place and having the lowest cost.[14] Also, amalgam restorations still have greater longevity than other restorative materials, show the best marginal integrity and have better performance in high caries risk patients.[15–18] Its disadvantages are not being tooth-colored, the higher probability of staining the surrounding tissue[19] and the need to create mechanical undercuts for macromechanical retention,[20] usually resulting in greater loss of tooth substance (although amalgam bonding systems exist),[21] besides the presence of mercury, which raises environmental and personnel safety concerns. Nevertheless, due to its durability and low cost,[22] a great number of people still have amalgam restorations in perfect functional conditions, and the role of the metallic constituents perfusion is of concern and has been the object of several studies,[23–25] some of which correlate amalgam mercury contamination with the onset of Alzheimer disease.[26]

There are many ways in which the mercury present in amalgam fillings can get into the human body: as immediate consequences of a dental restoration, mercury can be inhaled as a vapor or its debris ingested, and chemical reactions can occur between the amalgam and foods with subsequent ingestion; moreover, long-term migration from the amalgam in the dental structure to the bloodstream can be expected. The first two possibilities lead to ingestion of mercury in the metabolic process, while the last one leads to direct contamination of the bloodstream through diffusion processes that occur with time, causing a concern about aging amalgam restorations in older people.[27] Furthermore, in addition to the presence of mercury, the other metal constituents of the amalgam, which are not as harmful as mercury at low concentrations, can also diffuse to the surrounding dental tissue.

Zwicker et al.[28] recently reported above the normal mercury concentration in urine from a chosen group of individuals with dental fillings, raising concerns about the dental use of amalgam. Although the mercury levels found in the urine were considered too low to pose health risks, removal of amalgam fillings reduced the likelihood of self-reported symptom deterioration and increased the percentage of symptom improvement in comparison to people who retained their amalgam fillings.

Hoffmann et al.[29] investigated the mercury migration in human teeth using spatially resolved analysis by laser ablation inductively coupled plasma mass spectrometry (LA-ICP-MS). The authors found that the concentration of mercury diffused from amalgam fillings through the dental tissue decreases by six orders of magnitude in 2 cm, and they concluded that additional experimental research is necessary to elucidate the diffusion mechanism that may be taking place along the dentin tubules.[30] These tubules are hollow canaliculi that contain the processes of odontoblasts, which are cells located in the pulp tissue that are responsible for the secretion of collagen and non-collagenous substances, and for the formation and the maintenance of dentin. Around the odontoblasts and inside the tubules, in the periodontoblastic space, there is the presence of a liquor and the extracellular matrix, composed mainly by proteins, and non-calcified collagen fibrils are occasionally present. In this way, the dentin tubules extend across the entire thickness of dentin and are responsible for the diffusion of nutrients throughout dentin.[30]

Another study of the migration of the amalgam constituents into the dentin using X-ray fluorescence produced a concerning result, indicating a high Hg concentration in dentinal tubules and that there is direct contamination of the blood by mercury.[31]

Due to the inherent physical and chemical characteristics of dental tissues and of mercury, the migration study of this analyte into dentin is not a straightforward analytical chemistry task. The chemical constituents of the tooth tissue layers are not static and are tolerant to substitution by a variety of trace elements. It changes constantly with age, environment, diet and the presence or absence of demineralization.[32–34]

It must also be pointed out that the placement of restorative materials will induce a response in the tooth dentin–pulpal complex to some degree. A severe response can lead to pulp inflammation or even necrosis once ions from restorative materials can diffuse through the dentin tubules into the pulp tissue. In this way, it is important to choose a biocompatible restorative or lining material in order to protect the dentin–pulpal complex, mainly in deep cavities.[35]

Singh et al.[34] recently pointed out the importance of laser-induced breakdown spectroscopy (LIBS) for studying hard tissues (e.g., bone, teeth) and other calcified tissue material. The authors highlighted the role of major and minor elements present in bone and teeth, and reviewed most of the applications of LIBS for the trace analysis in calcified tissues with the main emphasis on the spatial mapping of elements.

Laser-induced breakdown spectroscopy is a powerful tool for direct analysis due to its inherent analytical capabilities for fast and simultaneous multi-elemental determinations. This spectroscopic method consists of using a laser for sampling (through ablation in solids and liquids) and simultaneously producing a high temperature plasma from any type of material with excitation/ionization of the species which emit their characteristic radiation.[36] This technique is considered very promising for analysis of samples of different fields, such as environmental, agronomic, biomedical and forensic, among others. In many cases, the chemical characterization of solid, liquid or gaseous samples is surprisingly quick and simple, without the use of reactants or waste generation.

According to Kaiser et al.,[37] compared to other laser ablation analytical techniques, LIBS can be considered simpler and generally more cost effective. Laser-induced breakdown spectroscopy can provide instantaneous signals directly related to the location at which a single ablation event occurred. In another way, mapping using laser ablation inductively coupled plasma optical emission spectrometry or LA-ICP-MS can present experimental drawbacks, once it involves sample dispersion into the ablation chamber and also during aerosol transport to the plasma torch. According to the authors, without considerable effort, the signal produced in the mass or optical spectrometer by laser ablation techniques could not be directly attributable to a specific location on the sample. These mapping problems are minimized when using LIBS, and although differences on laser–sample interaction have hindered the application of LIBS in studies involving different types of biological tissues, this has been changing by refinements on the technique involving new methods of sample preparation[38] and the use of shorter wavelengths into the ultraviolet (UV) (fourth harmonic of neodymium (Nd), nanosecond lasers),[39,40] which promote a less selective ablation. In order to reduce the matrix effects, the use of ultrashort (femtosecond) laser pulses can be recommended. In many cases, stoichiometric sampling can be achieved by using femtosecond lasers in the ablation process of LIBS methods.[36,41,42]

In this way, considering the advantages of the femtosecond laser-induced breakdown spectroscopy (fs-LIBS) and the conflicting results concerning the migration of ions from amalgam to dentin tissue, the aim of this work is to study the diffusion of mercury and the other amalgam elements into the dental tissue using the fs-LIBS technique.[41,43,44] This technique was chosen due to the less selective ablation promoted by the femtosecond laser pulses and the decreased dependence on the material matrix, which favors the determination of elements in different materials such as amalgam and dentin.

Experimental

The samples used in this work were obtained from 15 extracted human molar teeth, seven of which were deciduous teeth with full root reabsorption (samples D01–D07), and eight permanent teeth (samples P01–P08). All teeth had occlusal amalgam fillings, with no gap between the amalgam and the dentin, as could be seen under an optical microscope (60× magnification). They were provided by the Human Teeth Bank of the Dental School of the University of São Paulo, and their use was approved by the human ethics committee of the same institution. The teeth were free of caries and the amalgam restorations were restricted to the occlusal face, with a minimum thickness of 3 mm and without lining between the amalgam and the tooth when seen in periapical radiography.

The teeth were half-sectioned with an occlusal–cervical cut. For that, they were fixed in an acrylic board with wax, mounted in a precision *xyz* translation stage and then cut by a refrigerated diamond saw (ACCUTOM 50, Struers). The occlusal–cervical cut was performed in such way that it was possible to see the crown, the amalgam filling and the dentin up to the pulp. Two parallel cuts were performed in each tooth, providing 1 mm-thick samples. These samples were carefully rinsed with distilled water in an ultrasonic bath in order to remove residues, and then individually fixed in microscope slides with wax. In all the samples a dark region close to the amalgam fillings was clearly observed. The amalgam restorations studied may have variations in the alloy composition, and no information about the teeth or the restoration ages was available since the specimens came from a human teeth bank.

To perform the fs-LIBS measurements, an amplified Ti:sapphire laser system was used. This system was composed of a Rainbow (Femtolasers) main oscillator seeding an Odin (Quantronix) CPA amplifier. This system generated 30 fs pulses, centered at 800 nm, in a 90 Hz repetition rate train of pulses with energies ranging from 195 μJ to 270 μJ. The pulses were focused by a 75 mm lens, and the samples were positioned 1 mm before the focal point to avoid air breakdown, leading to a 67 μm spot size radius at the sample surface. The laser beam incidence angle on the sample was 45° to allow collection of the plasma emission along its longitudinal axis (normal to the sample face), as shown in Figure 1, increasing the collected signal. This geometry resulted in a 4.5×10^{13} W cm^{-2} intensity at the sample surface for the maximum laser output energy.

The plasma emission was collected and collimated by fused silica lenses (Ø25.4 mm). A 5 cm focal length lens was used to collect the plasma emission, which was focused on the spectrometer optical fiber by a 12.5 cm focal length lens to match its numerical aperture (Figure 1).

The plasma emission was analyzed by an Echelle spectrometer (ESA 3000, LLA Instruments) with a 25 cm focal distance and f# 1/10, linear dispersion per pixel of 0.005 nm (200 nm) and 0.019 nm (780 nm), resolution with respect to one pixel ($\lambda/\Delta\lambda$) of 40 000, and spectral range from 200 nm to 780 nm. Wavelength calibration was performed by using electrodeless cathode Hg lamps (EDL II System, Perkin Elmer). Its detector is an ICCD camera, (Kodak KAF 1001) with 1024 × 1024 pixels and a pixel size of $24 \times 24\,\mu\text{m}^2$, and a microchannel plate as image intensifier. The dark current is automatically subtracted at each spectral measurement. Each spectrum acquisition was initiated by a trigger signal generated by the laser, sent through a timing unit (DG535 delay generator from SRS).

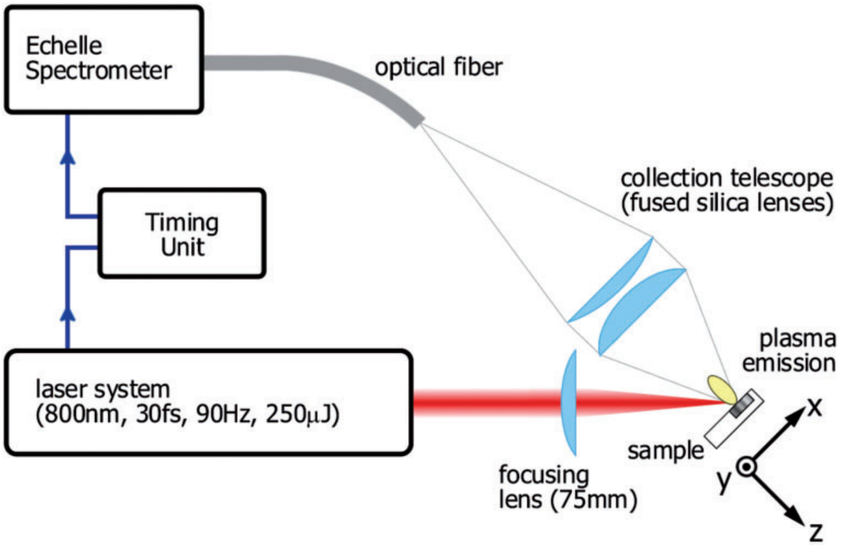

Figure 1. Experimental setup scheme.

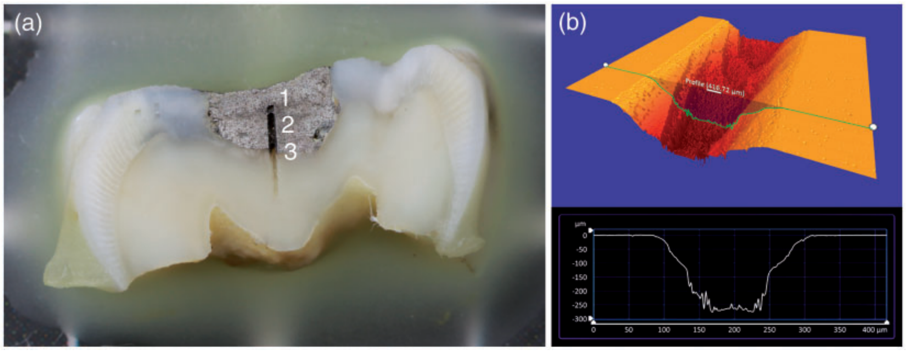

Figure 2. (a) Deciduous tooth with three regions indicated: (1) amalgam; (2) amalgam/tooth interface and (3) tooth, with the ablation path starting in (1) and proceeding to (3). A gray region can be observed in the dental tissue around the amalgam filling, indicating that the metallic elements migrated into this region. (b) Topographic map of the ablation path in the tooth region, showing an ablation depth greater than 250 μm.

A pilot experiment was conducted to determine the experimental parameters that optimized the spectra acquired (background minimization and signal to noise ratio). These were found to be a 90 Hz laser repetition rate, 60 ns delay from the laser pulse and a 150 ns integration window, with 5 s acquisition time. These parameters were used throughout the experiments described here.

The samples were mounted in a computer controlled *xyz* translation stage, with their faces in the vertical *x*–*y* plane (Figure 1). The sample scanning was performed in this plane so that the plume of ejected material would move away and fall outside the sample, minimizing contamination of the sample surface. An argon flow across the sample surface assisted in moving away the ejected material. Each LIBS spectrum is the accumulation of 450 measurements (5 s acquisition at 90 Hz repetition rate), while the translation stage performed periodic movements with different frequencies in the *x* and *y* directions, covering an area of $100 \times 100\ \mu m^2$; to obtain each spectrum, the translation stage started this movement and the laser hit the sample surface, and only after 5 s the spectrometer started acquiring the data. In this way, the surface layer was ablated before the measurements, not contributing to the final spectrum. After a spectrum was acquired, the translation stage was moved laterally for 100 μm, so a fresh area of the sample was irradiated by the laser and a new spectrum was obtained. For each tooth the first measurement was done in the amalgam and the lateral displacements were repeated moving the laser incidence point through the amalgam/tooth interface, into the dental tissue for at least 1 mm, defining a path along which the spectra were measured, as shown in the analyzed sample depicted in Figure 2a. It is important to note that the ablation path followed, roughly, the direction of the dentin tubules that run from

Table 1. Amalgam constituent element emission lines measured, selected in a pilot experiment.

Element	Mercury	Silver	Copper	Tin	Zinc
Emission line (nm)	253.652 (I) [900 000]	224.641 (II) [900 000]	223.008 (I) [2500]	283.998 (I) [13 000]	202.548 (II) [500]
	296.728 (I) [3000]	241.319 (II) [470 000]	224.700 (II) [1000]	286.332 (I) [10 000]	206.200 (II) [500]
	398.393(II) [10 000 000]	243.778 (II) [450 000]	324.754 (I) [10 000]	303.412 (I) [10 000]	213.856 (I) [800]
	404.656 (I) [12 000]	328.069 (I) [55 000]	327.396 (I) [10 000]		330.258 (I) [800]
	435.833 (I) [12 000]	338.289 (I) [28 000]			334.502 (I) [800]
		520.908 (I) [1000]			481.053 (I) [400]

The Roman numerals between parentheses indicate the ionization state of the element, and the numbers inside brackets are the line relative intensities obtained from the NIST Atomic Spectra Database.[45]

the enamel–dentin junction to the pulp chamber, and comprised about 20 sampling points. The upper part of Figure 2b exhibits a topographic measurement of the ablation path in the dentin of this tooth, performed by a ZeGage Optical Surface Profiler (Zygo), showing that a volumetric sampling was done; the lower part plots the profile show in green in the upper part, and it can be seen that the depth of the ablation is over 250 μm, being the result of the surface cleaning and the volumetric sampling. This deep ablation minimizes, in the final spectrum, the contribution of an eventual surface contamination remaining after the surface laser cleaning.

In the pilot experiment, the emission lines shown in Table 1 were chosen in order to determine the migration of these elements into the dental tissue. The zinc emission lines shown in Table 1 are the most intense ones, but these lines were not observed in the fs-LIBS spectra acquired.

The measured emission line areas were plotted as a function of the position along the ablation path in the sample, and these data were fitted by an error function:[46]

$$A(x) = \frac{A_0}{2} erf\left(1.81239\frac{x-\xi}{\delta}\right) + b \tag{1}$$

where x is the position along the ablation path, A_0 is the line emission area in the amalgam, ξ a displacement from the origin and b is a constant. The error function (*erf*) represents a rapid change in amplitude from a high value plateau to a low value plateau, and the parameter δ is the distance at which the function value drops from 90% to 10% of its maximum value, and was considered to be the penetration depth of the element into the dental tissue.

The calculations were repeated for all the different emission lines for each element in order to check the consistency of the values obtained for the penetration depth δ.

Results and discussions

Figure 3 exhibits typical spectra of the three different regions (amalgam, interface and dentin) as defined in Figure 2. From these spectra, a wealth of qualitative information can be obtained: it can be clearly seen that these spectra are different, and while the metallic elements (Ag, Hg, Cu and Sn) emission lines predominate in the amalgam spectrum (a), dental constituent ones, Ca and Mg, dominate the tooth spectrum (c), providing a fingerprint of each material. All these emission lines can be seen in the interface spectrum (b) demonstrating that the LIBS method can simultaneously detect the presence of metals in the dental tissue, among the tooth constituent elements. This is possible due to the less selective ablation character of the femtosecond laser pulses (when compared to nanosecond lasers), which was used as an advantage to probe metallic elements in a blend (amalgam) and also in composites/dielectrics (dental hard and soft tissues). It is worth noting that the Hg emission lines are not very intense, but they rise above the noise and can be readily measured. Zinc emission lines (Table 1) were not observed, and this could have been caused by low quantities (under 1% m/m), or complete absence, of this element in the samples, because many professionals prefer to use amalgams without zinc. Alternatively, this could have happened because our experimental set-up did not have enough energy to excite and measure transitions that are located well above the ground state.[45] This is also likely to be the cause for not observing any of the phosphorus emission lines (main ones at 213.618, 214.914, 253.560, and 255.325 nm) because it is one of the major components of the tooth.[47]

Figure 4 shows a fragment of the three spectra depicted in Figure 3, highlighting the Hg(I) 253.652 nm emission line. It can be seen that the amalgam is the main source

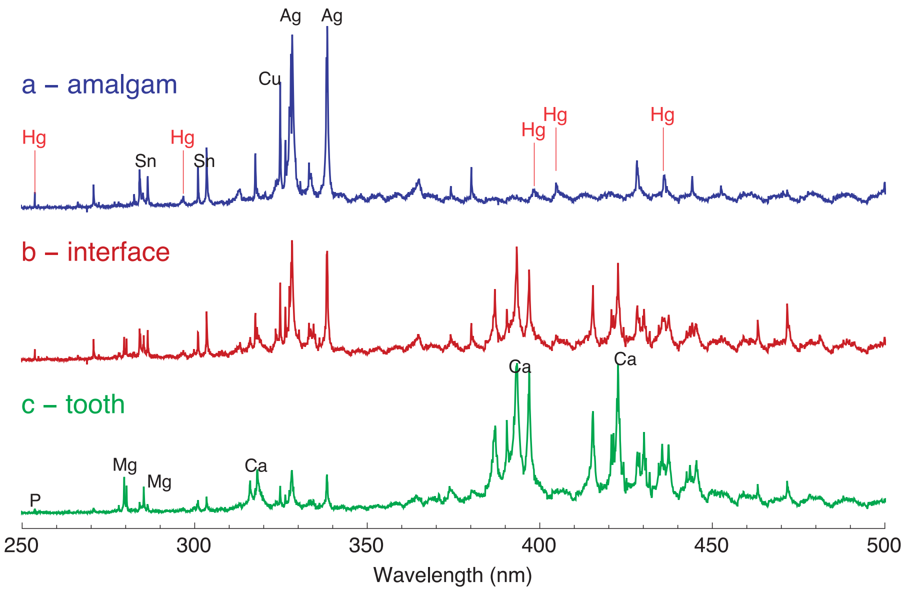

Figure 3. Spectra representative of the three regions (amalgam, interface and tooth). The amalgam and the tooth spectra were measured 400 µm away from the interface. The mercury emission lines measured in this work are highlighted in the amalgam spectrum.

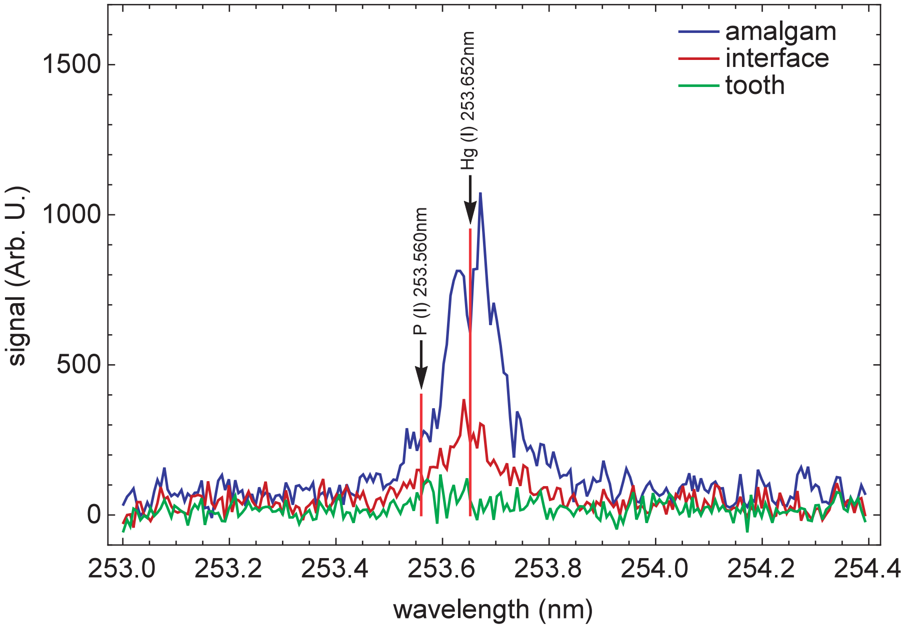

Figure 4. Fragment of an emission spectrum obtained by femtosecond laser-induced breakdown spectroscopy in a tooth. Signal data accumulated from 450 laser shots with 260 µJ per pulse; delay time: 150 ns; integration time: 60 ns.

of mercury, and that the corresponding emission line is not interfered with by the phosphorous atomic line at 253.560 nm.

Figure 5a–c presents the Hg emission lines at 296.728, 404.656 and 435.833 nm, respectively, in which strong background emissions could be observed. In these spectra, the red vertical line marks the mercury emission line wavelength taken from the NIST Database,[45] and the dashed curve is the emission background that was obtained fitting a polynomial function to data outside the peak (on its wings). The shadowed region represents the net integrated area that was taken to be proportional to the element concentration.

Figure 5d shows an Ag emission line that exhibits a dip due to self-absorption, indicating that this transition ends in the ground state, which agrees with the literature,[45]

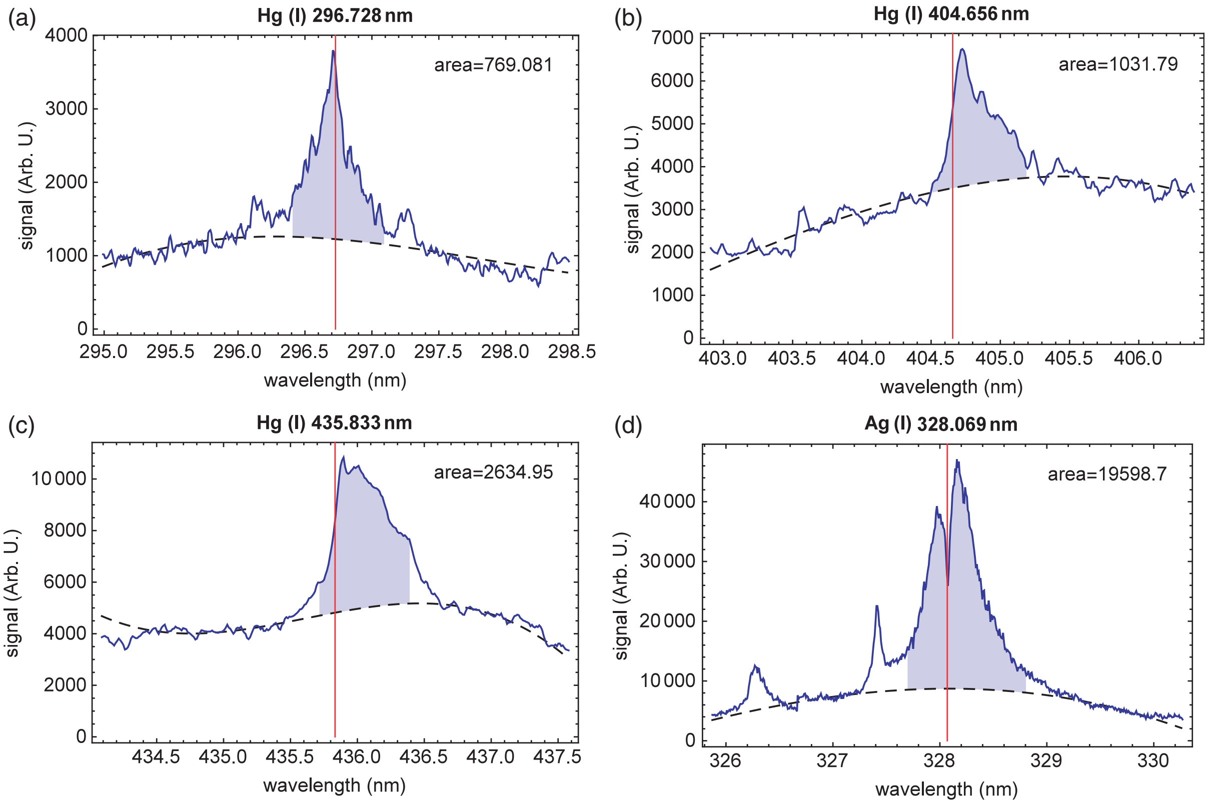

Figure 5. Mercury (a, b, and c) and silver (d) emission lines interfered with by other element emissions. The red vertical line indicates the center of the emission line (Table 1), the shadowed region is the measured area that was associated with the emission line, and the dashed line is the subtracted background.

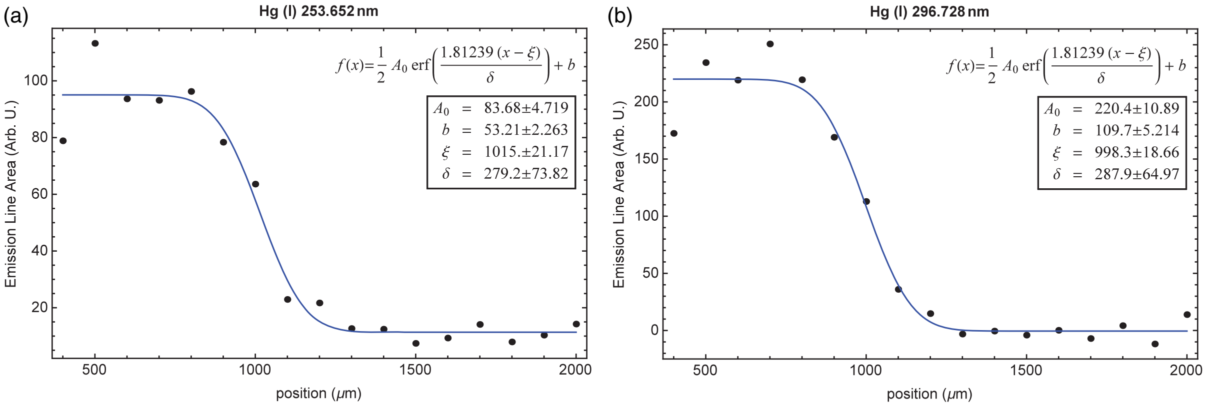

Figure 6. (a) Area of the Hg(I) 253.652 nm emission line as a function of the position measured along the ablation path on sample P02, and (b) the same for the 296.728 nm line. Each data set is fitted by an error function that provides the penetration depth δ.

where there is a population density capable of absorbing the emitted light. This is also the origin of the dip observed in the Hg transition[45] shown in Figure 4.

Figure 6 exhibits the Hg(I) 253.652 and 296.728 nm lines area dependence on the position along the ablation path on sample P02 (points), together with fittings by Eq. 1 (solid line). The fittings provide the penetration depths δ, which are 279.2 μm and 287.9 μm for the 253.652 and 296.728 nm lines, respectively. In this particular case, a very good agreement is observed, corresponding to a difference under 3%. Generally, the element penetration depth values obtained from different lines in a tooth differed by 10–20%, showing the self-consistency of the used method. It can also be observed in Figure 6 that across the penetration depth δ the element concentration drops for an order of magnitude.

Table 2. Elements penetration depth in deciduous teeth samples (uncertainties are represented by ±1 estimated standard deviation).

	Penetration depth δ (μm)						
	D01	D02	D03	D04	D05	D06	D07
Hg	159 ± 20	186 ± 19	157 ± 28	151 ± 13	93 ± 17	460 ± 53	172 ± 16
Ag	153 ± 10	172 ± 21	200 ± 21	134 ± 8	57 ± 15	456 ± 39	222 ± 15
Cu	154 ± 13	144 ± 35	212 ± 25	142 ± 8	72 ± 10	429 ± 38	174 ± 17
Sn	–	165 ± 24	212 ± 32	140 ± 7	86 ± 21	396 ± 42	246 ± 34

The number of repetitions for each element is the number of lines indicated in Table 1.

Table 3. Elements penetration depth in permanent teeth samples (uncertainties are represented by ±1 estimated standard deviation).

	Penetration depth δ (μm)							
	P01	P02	P03	P04	P05	P06	P07	P08
Hg	124 ± 21	126 ± 34	118 ± 16	171 ± 10	204 ± 39	237 ± 23	163 ± 14	355 ± 24
Ag	128 ± 8	148 ± 54	111 ± 12	175 ± 6	209 ± 25	246 ± 21	188 ± 13	326 ± 26
Cu	138 ± 8	168 ± 52	115 ± 18	182 ± 9	228 ± 24	294 ± 22	202 ± 38	394 ± 37
Sn	133 ± 7	189 ± 9	96 ± 24	181 ± 12	237 ± 44	188 ± 32	166 ± 12	638 ± 56

The number of repetitions for each element is the number of lines indicated in Table 1.

This procedure was repeated for each sample, for the lines indicated in Table 1 (except for zinc), and an element penetration depth in the sample was taken to be the weighted average of the measured δ values based on these emission lines (six lines for Ag, four lines for Cu, five lines for Hg and three lines for Sn). In this procedure, each emission line was measured only once per sample, and the number of lines shown in Table 1 determines the repetitions for each element. The penetration depths obtained in this way are shown in Table 2 for the deciduous teeth and in Table 3 for the permanent teeth.

From the values on Table 2 and Table 3 it can be seen that the elements penetration depth values in each tooth are usually consistent, indicating that all the metals diffuse together into the dental tissue. Nevertheless, a few discrepancies exist: in sample P08 the tin penetration depth is almost twice the depth for the other elements; in sample P06 it is sensibly smaller than for the other elements, and could not be determined in sample D01; for the copper, δ is bigger than for the other elements in sample P06. Also from these tables it is possible to obtain the mercury mean penetration depths in the samples, and their values are shown in Table 4. It can be seen that, on average, mercury diffuses further in permanent teeth than in deciduous teeth, but there is one caveat: the teeth and the restoration ages are not known, and it is expected that an older restoration produces further diffusion of its elements into the surrounding material, and this should be taken into account in future studies to reinforce this conclusion. Considering this, the permanent teeth sample P08 exhibits deeper penetration depths than in any other permanent samples, and this can indicate an older restoration from which the metals had more time to diffuse into the surrounding dental tissue. This behavior is similar in deciduous sample D06, which has the deepest Hg penetration. Nevertheless, the penetration depth is usually of a few hundred microns; in this way, considering the possibility of staining due to the amalgam restorations, as well as the need for protecting the dentin–pulp complex, it is not recommended to make amalgam restorations in deep occlusal caries that are less than 1 mm away from the pulp tissue. In fact, independent of the restorative material used, deep cavities with minimal remaining dentin thickness (equal to or less than 0.5 mm) require the presence of indirect or direct capping materials or the presence of adhesive liners in order to preserve the pulp vitality.[48,49]

Table 4. Mercury average penetration depth in deciduous and permanent teeth.

Hg penetration depth (δ)	Deciduous teeth	Permanent teeth
	(157 ± 7) μm	(173 ± 6) μm

We have measured the metal atoms diffusion into the dentin in a direction roughly parallel to the direction along the crown to the pulp chamber, the same at which the tubules are aligned, and their penetration depths are similar for the four elements studied (Hg, Ag, Cu and Sn), being of the order of hundreds of microns. As a consequence of these results, we hypothesize that the diffusion mechanism could be similar for all these elements, and that it would occur mainly through liquid transport (dragging) inside the dentin tubules. Dental restorations, which have an area of several mm^2, are in direct contact with at least tens of thousands of dentinal tubules.[50] This corresponds to an effective area of contact between the amalgam and the dentinal tubules fluids (mainly composed of albumin, transferrin, tenascin, blood plasma and proteoglycans) of approximately 10% of the total amalgam area of contact. This favors the diffusion process by fluid dragging inside the tubules over diffusion into a solid (dentin), which strongly depends on the solubility of the ion in the solid matrix and is short-ranged.[51] Moreover, the hundreds of microns' penetrations depth measured could not have been reached through diffusion into a solid in a few years at 36 °C, reinforcing the liquid dragging hypothesis. The rapid disappearance of mercury from the urine of patients who had fillings removed[28] reinforces our hypothesis, once the much slower diffusion in solids would not eliminate the Hg from the urine in a short time.

The literature reports that the phases formed and the mechanisms of corrosion of amalgam are different and depend on the alloy chemical composition. According to Von Fraunhofer and Staheli,[52] the formation of corrosion products should be preceded by metal dissolving to form ions in solution. These ions may react with the electrolyte or other corrosion products, and the initial main reactions that can occur are:

$$2\mathrm{Ag}^+ + 2\mathrm{OH}^- \rightarrow \mathrm{Ag_2O} + \mathrm{H_2O}$$

$$\mathrm{Sn}^{2+} + 2\mathrm{OH}^- \rightarrow \mathrm{SnO} + \mathrm{H_2O}$$

$$\mathrm{Sn}^{2+} + 4\mathrm{OH}^- \rightarrow \mathrm{SnO_2} + 2\mathrm{H_2O}$$

The most corrosion-prone of all amalgam components is the Sn-containing phase, which is frequently detected in the studies that involve amalgam corrosion and was shown in the present study. Considering the release of Hg from amalgams, other studies suggested the possibility of developing an oxide layer that reduces the subsequent dissolution of the various metal components.[53,54] The pH of the surrounding tissue, as well as the presence of secondary caries,[55] the marginal seal, the restoration age and the presence of reparative dentin, are characteristics that interfere in the corrosive process of amalgams. In this present study, considering that teeth provided from a human teeth bank were used, the samples were most probably submitted to different clinical conditions that influenced the liquid transport of metallic ions through the dentinal tubules. In spite of all these differences, the penetration depth is very similar for all the elements in the same tooth, in both the permanent and the deciduous teeth. These results contradict a similar study carried out by X-ray fluorescence imaging,[31] which established that different metals have different penetration depths into the dental tissue, but in that case the diffusion was measured in a plane perpendicular to the tubules and, consequently, transversely to the fluids movement direction. In this case, there is no liquid transportation and, probably, the predominant mechanism is the diffusion of atoms in a solid, which depends on the solid atomic arrangement, the diffused element size and electronic configuration and on the tooth temperature. Nevertheless, even when the migration occurs along the direction of the tubules, the Hg diffuses only a few hundred microns, showing that if the restoration is shallow (not coming close to the pulp) the blood contamination will be kept at safe health levels.[28] This is reinforced by our results being consistent with those of Hoffmann et al.,[29] which show, although using a lower spatial resolution than in the present work, that in the 1 cm range the Hg diffusion drops by three to four orders of magnitude.

Conclusions

Femtosecond laser-induced breakdown spectroscopy is a technique that, due to its ablation with low dependence on the material matrix and small ablation thresholds, allowed us to identify spatially the diffusion of metallic elements from amalgam restorations into the surrounding dental tissue. The presence of the metal constituents in the dentin was assessed by the fs-LIBS method, and its spatial distribution was determined. The agreement in the results obtained from different emission lines for each element ensures that the method is valid and can be performed with any elemental line.

We have not determined the absolute concentrations of the elements, but this is not meaningful in the present work, in which only the relative element quantities in a tooth are important. This technique allowed us to measure the penetration depth of the main constituents of amalgam restorations into the dentin, in deciduous and permanent teeth. For each element, the penetration depth values were averaged over several emission lines, and we have found that the behavior of the metal diffusion into the dentin can be described by an error function that has a transition region characterized by a penetration depth δ. It was possible to determine this penetration depth for mercury, silver, copper and tin. We also observed a deeper migration of these elements on permanent teeth when compared with deciduous teeth. This penetration depth, which is usually of a few hundreds of microns, represents a drop of one order of magnitude in the element quantity. An important fact is that the measurements were made along the dentin tubules from the amalgam restoration to the pulp direction, therefore any transport of amalgam constituents to the pulp by the liquids in the tubules is considered an important part of the diffusion process. The values obtained for the elements penetration within each tooth are usually self-consistent, indicating that the metals diffuse together into the dentin either for short (sample D05) or long (samples D06, P08) distances, although these have similar values of a few hundred microns. The teeth and the restoration ages are not known, so it is not possible to estimate the diffusion speed of the amalgam elements into the dental tissue. This can be the subject of a future study, but it is difficult to obtain samples with both these ages, mainly the restoration age, known. Nonetheless, we can conclude that shallow restorations will keep the Hg levels in blood at safe levels due to the rapid decay of mercury concentrations in less than a couple of hundred microns, which can be circumvented in the preparatory procedures and the technique definition.

The emission lines corresponding to other elements such as calcium, sodium, magnesium, among others, were also observed, configuring the fs-LIBS technique as a valuable tool to map the distribution of endogenous and exogenous tooth elements, with a spatial resolution that can be brought under 100 μm.

Conflict of Interest

The authors declare that there is no conflict of interest.

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