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Methodological strategy for the analysis of human dental enamel by LA-ICP-MS

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Abstract

The application of LA-ICP-MS to human dental enamel usually follows a methodological procedure that starts with the vertical sawing of the tooth in order to obtain a flat surface. However, the inner enamel can be accessed in archaeological teeth that are fractured for natural, taphonomic reasons, which reduces further damage to archaeological specimens by cutting them. This paper analyzes the differences in the counts of trace elements by laser ablation–inductively coupled plasma–mass spectrometry between a cut and broken surface from the same teeth. Results show that the two surfaces do not produce statistically different readings, which extends the possibility to apply this methodological procedure to archaeological remains that cannot be sectioned, and therefore reducing the amount of damage effected to important specimens.

Keywords: Laser ablation; Human teeth; Inner enamel; Trace elements

1. Introduction

During the last decade or so, laser ablation–inductively coupled plasma–mass spectrometry (LA-ICP-MS) has seen increasing use as an analytical tool to detect trace element concentrations in a variety of materials. In archaeology, LA-ICP-MS has been used on ceramics, glass, metals, obsidian and semiprecious stones (Speakman and Neff, 2005). Overall, ‘‘LA-ICP-MS has become one of the most exciting new fields of research in material science’’ (Speakman and Neff, 2005:1). Its ability to target specific areas has been useful in the analysis of temporal variation in the elemental composition of human teeth (Cox et al., 1996; Budd et al., 1998; Lee et al., 1999; Lochner et al., 1999; Dolphin et al., 2005).

Human teeth develop over time (Hillson, 2002), with appositional layers deposited in onion-like fashion (Kang et al., 2004). Dental enamel is first laid down at the dentin-enamel junction just below the cusps or incisal margin of the crown, and deposition of enamel gradually continues toward the cemento-enamel junction that at the time of completion of the crown formation will represent the boundary between crown and root. This means that the earliest enamel to form can be found at cusp level and the latest toward the root. The enamel is very resistant to diagenetic contamination; nonetheless, the external surface may incorporate contaminants from the burial environment.

At present, several features distinguish laser ablation analyses on human remains from other archaeological and geological applications. One problem is that matrix-matched hydroxyapatite standards so far have not been developed to calibrate elemental concentrations in enamel or bone. Since hydroxyapatite constitutes about 97% of human dental enamel (Hillson, 2002), the development of a standard for hydroxyapatite is an important goal of an ongoing research. Matrix matching of standards to actual specimens is important, because ablation volumes and laser–sample interactions can affect the ICP-MS signals in unpredictable ways. In practice, however, many of these effects can be estimated using samples

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of known concentrations, and we believe that proper standard correction factors enable us to acquire useful compositional data in the absence of matrix matching. Another set of problems relates to sample preparation, and this is what concerns us in the present paper.

To avoid contamination and in order to expose the inner enamel, the method of sampling human teeth consists of sectioning the tooth along its vertical or horizontal axis. This is usually accomplished with a low speed diamond blade (or similar), and the tooth is usually treated chemically or simply washed and sonicated with distilled, deionized water to remove potential contamination from the sawing process (Cox et al., 1996; Budd et al., 1998; Lee et al., 1999; Lochner et al., 1999; Kang et al., 2004; Dolphin et al., 2005). This approach produces a homogeneous, flat surface, but it requires both an Isomet low speed blade (which not every lab may have on hand), and more importantly, the permission to cut or otherwise alter the tooth. While LA-ICP-MS is essentially non-destructive in that it leaves an imperceptible mark on the ablated surface, sectioning of the tooth is obviously destructive and may reduce the chances that permission for analysis will be granted by museums or governmental institutions. Even though very little mass is removed by the diamond blade, gluing the two halves back together after the analysis does not restore the tooth to its original size and morphology.

Like bones, archaeological teeth may fracture as a result of taphonomic causes. This may expose the inner enamel without the need for sawing. Therefore, in an effort to reduce damage to human archaeological remains and to increase the applicability of LA-ICP-MS to museum specimens that may not be available for destructive analysis, the present study aims to investigate whether the results obtained from an artificially sectioned tooth differ significantly from those obtained from the inner surface of a tooth that was broken naturally in a post-depositional context. The results might differ because an uneven surface may induce variation in the interaction between the laser beam and the surface, with a consequent change in the process of vaporization and transportation of sample material to the ICP-MS.

2. Materials and methods

The instrument set-up used in this study consisted of a New Wave UP213 laser ablation system coupled to a GBC Optimass 8000 time-of-flight (TOF) ICP-MS. The TOF-ICP-MS separates analytes according to mass/charge ratio on the basis of elapsed time to reach the detector (heavier ions arriving after lighter ions). This approach to mass spectrometry dramatically reduces the time needed for data acquisition and provides analytical precision that is independent of the number of analytes being monitored.

In order to simulate a naturally broken enamel surface, 12 archaeological teeth from various sites in Yucatan (México) were initially broken mechanically with pliers, positioning the two blades along the vector that crossed one or two adjacent cusps, so that the fracture would expose the enamel below them. This was a simple and fast process that required only a few seconds and very little power. The teeth used in this study are part of a long term project that aims to use LA-ICP-MS to detect variability in trace elements from dental enamel as determinants of individuals of foreign origin (Cucina, 2005a,b; Cucina et al., 2005).

In most cases, the broken surface appeared fairly regular and flat, in particular when the tooth was solid and sturdy. At this point, one half of each tooth was mounted on an Isomet low speed chuck with the broken inside facing parallel to the diamond blade in order to remove the thinnest possible section and obtain a flat surface, which would resemble that of an artificially cut tooth. The sectioned half of the tooth was then cleaned with distilled water to remove possible contamination from the saw. This process yielded two homologous surfaces of the same tooth, one broken and one cut separated by a distance of less than 1 mm (the equivalent of the portion cut out by the blade). Such closely spaced portions of the same tooth can be expected to have very similar chemical signatures.

The two halves of each sample were placed in the laser chamber, one next to the other with the inner surface as horizontal as possible. The laser chamber was large enough to hold four pieces (two teeth) at the same time, along with SRM614 and SRM612 glass standards used for calibration. A raster drawn on every surface was pre-ablated to remove potential contaminants on the surfaces. The raster was drawn in the very same location on the crown of each half of each tooth in order to limit error introduced by analyzing different parts of the crown that may potentially reflect different moments in life (Budd et al., 1998; Lochner et al., 1999; Kang et al., 2004; Dolphin et al., 2005). After the pre-ablation pass, data collection was initiated on the ICP-MS. Data from the two glass standards were recorded before and after each set of samples in the laser chamber, in order to provide precise calibrations that compensate for instrumental drift. Each raster provided three sets of five readings for each element, which were then averaged to get the final signal intensities. Data were calibrated to parts per million using the NIST glasses, with ^{43}Ca as an internal standard (see Dolphin et al., 2005). In most cases, the SRM614, with concentrations of most elements less than 1 ppm, was used for calibration. As mentioned previously, a further improvement to our method would be to fabricate a series of hydroxyapatite standards with graded concentrations of trace and minor elements found in teeth. Normalized data for the elements from the broken and cut surfaces of each tooth are presented in Table 1.

3. Results

Table 1 lists the normalized element concentration from the two halves of each tooth.

The mean and standard deviation of the 13 elements from the 12 teeth are listed at the bottom of each column. Elements whose concentration was below 1 ppm were not considered for further elaborations. In the case of Ba and Hg, one or two individuals (teeth 1 and 2) were below detection level. Table 2 lists the results obtained from the two-tailed *t*-test for dependent samples performed using SPSS 11.5 software.

Table 1
Concentration of elements from the broken and cut surfaces of the 12 teeth analyzed

Tooth	Na		Mg		Al		P		Cl	
	Broken	Cut	Broken	Cut	Broken	Cut	Broken	Cut	Broken	Cut
1	3891.3	5122.2	1459.0	1837.1	0.8	0.5	184675.1	190071.4	3.7	2.7
2	4440.3	5425.1	1323.4	1250.5	0.8	0.8	189782.9	199410.1	3.5	2.4
3	4213.2	4337.9	1795.7	1730.7	7.7	8.3	192694.0	207859.2	2.6	3.0
4	4280.9	4813.4	1448.1	1693.3	2.2	5.9	210299.5	212014.0	3.2	3.2
5	6306.0	6796.6	1722.8	1686.9	0.4	0.3	211879.6	209449.5	2.8	2.4
6	5521.6	5983.9	1540.1	1285.2	1.8	1.3	200427.6	212081.9	3.4	3.0
7	5519.4	5797.5	4279.7	2764.7	12.1	5.9	184638.8	208217.8	4.1	4.1
8	4932.6	4602.4	2001.9	1614.8	6.8	1.6	198116.8	190924.5	3.4	2.3
9	5258.4	4746.6	2546.1	2335.3	1.5	1.0	196175.6	187473.3	3.1	2.3
10	4400.0	4255.3	2118.3	2017.6	3.4	0.5	191394.4	198802.2	2.8	3.4
11	4150.1	5581.9	2302.9	3203.8	2.6	0.9	193220.9	190002.1	3.8	2.5
12	4576.2	4489.6	9876.0	2920.7	0.4	0.2	214822.6	202612.4	3.0	4.0
Mean	4790.8	5162.7	2701.2	2028.4	3.4	2.3	197344.0	200743.2	3.3	3.0
SD	722.0	772.2	2395.4	639.4	3.6	2.8	10221.3	9286.8	0.4	0.6

Tooth	Sc		Ti		Zn		Sr		I	
	Broken	Cut	Broken	Cut	Broken	Cut	Broken	Cut	Broken	Cut
1	4.24	3.99	32.72	30.98	5.05	6.25	291.02	214.47	6.21	8.07
2	4.10	3.01	29.87	29.19	6.66	7.98	124.89	123.27	7.94	13.49
3	4.28	4.11	39.37	45.68	6.63	9.60	522.00	662.17	13.69	14.43
4	4.33	4.40	43.93	41.98	6.49	6.89	341.52	145.31	5.18	0.72
5	3.94	3.58	51.65	45.24	7.09	8.35	228.80	232.55	4.84	6.84
6	3.74	3.59	36.80	40.29	7.97	8.82	438.35	299.97	9.04	7.45
7	4.27	4.00	38.49	45.27	9.77	13.95	701.61	179.75	8.28	7.95
8	4.14	3.70	38.23	34.07	5.20	2.45	538.32	646.67	8.36	10.95
9	4.32	3.99	36.56	33.58	10.43	5.56	268.90	283.59	6.89	10.71
10	4.49	4.31	30.85	35.53	4.22	4.91	281.19	174.81	7.91	6.53
11	4.24	4.24	33.40	37.25	8.16	6.71	193.79	216.91	20.26	13.30
12	3.69	4.28	33.20	37.08	9.22	6.13	249.93	113.23	28.05	10.84
Mean	4.15	3.93	37.09	38.01	7.24	7.30	348.36	274.39	10.56	9.27
SD	0.24	0.40	6.08	5.68	1.94	2.84	168.17	186.52	6.94	3.83

Tooth	Ba		Hg	
	Broken	Cut	Broken	Cut
1				
2				
3				
4	1.15	0.85	8.51	53.69
5	0.09	0.14	11.14	7.25
6	2.74	1.68	36.58	19.31
7	4.27	1.10	5.25	23.54
8	1.03	1.30	6.07	39.20
9	0.14	0.17	4.25	11.66
10	0.62	0.43	21.23	10.51
11	0.17	0.14	5.31	18.18
12	1.75	0.09	5.63	10.24
Mean	1.50	0.99	10.78	20.84
SD	1.42	1.21	10.43	14.75

4. Discussion

The size and morphology of a surface may interfere with the laser and the vaporization process that takes place in the laser chamber. Irregular surfaces are known to produce slightly different results. Lee et al. (1999) note that craters produced by the ablation of the dental enamel they were analyzing were not symmetrical, which may have been due to imperfect leveling of the samples.

Trejos and Almirall (2005) performed a test on SRM 610 and SRM 612 glass standards in order to examine potential differences in the results from LA-ICP-MS analyses when the size and shape of the samples might be of concern (glass in their case). The starting hypothesis was that analysis of glass pieces for forensic purposes might be affected by size and shape, because of differences in the way the glass dissipates heat. Sample shape was also implicated because of the alterations that might be induced in the interaction between the laser and the

Table 2
t-test for dependent samples between the elemental concentration in broken and cut surfaces

	Difference between means	SD of difference	*t*	Degree of freedom	*p*
Na	−371.8	610.9	−2.1	11	0.059
Mg	672.7	2056.8	1.13	11	0.281
Al	1.09	2.64	1.4	11	0.179
P	−3399.2	10683.7	−1.1	11	0.294
Cl	0.34	0.76	1.55	11	0.149
Sc	0.21	0.38	1.93	11	0.08
Ti	−0.92	4.41	−0.72	11	0.484
Zn	−0.05	2.61	−0.07	11	0.940
Sr	73.9	173.8	1.47	11	0.168
I	1.28	6.09	0.72	11	0.482
Ba	0.41	1.09	1.32	9	0.210
Hg	−10.06	19.01	−1.67	9	0.129

surface and the consequent variation in the process of vaporization of the glass sample. According to the results of the authors on fragments of the very same SRM 610 and SRM612 standards, size and shape do not represent a barrier to the performance of LA-ICP-MS, at least for specimens that are up to 0.1 mm in size (Trejos and Almirall, 2005).

The test performed on the 12 archaeological teeth is consistent with the results reached by Trejos and Almirall (2005), despite the different materials analyzed. None of the *t*-tests for dependent samples were significant; only Na even approached (but did not reach) the 0.05 level of significance ($p = 0.059$). Moreover, as data from Table 1 show, a broken surface did not produce any regular pattern in which concentrations were constantly lower or higher than the ones recorded from a cut, flat surface, or any trend that might relate to atomic mass or to the overall concentration of the element. All the fluctuations appeared to have been random throughout the analysis.

The lack of statistical differences and patterns between broken vs cut surfaces may have something to do with the histological structure of human dental enamel. Enamel consists of prisms running throughout the thickness of the enamel from the dentino-enamel junction to the external surface of the crown. Prisms (each one approximately 5 μm wide) are tightly packed and are separated by the so-called interprismatic enamel (Hillson, 2002). When pressure is applied, the fracture vector likely runs along the prisms, in a rather linear direction (although no test was performed in this study to prove this hypothesis). Although the overall gross morphology of the fractured surface may not be flat and may change direction, it is composed of several almost flat small surfaces. In this context, laser ablation turns out to be extremely useful because it has the capability to target very small areas of the surface for analysis, especially when a raster is confined to a restricted area, or when the enamel is spot-ablated. Although the broken surface is clearly not perfectly flat, focusing with the video camera mounted on the laser chamber permits the analysis to be restricted to areas that are completely in focus, which means that the visible area is fairly horizontal. It is worth pointing out that the lens' depth of field at high magnification is extremely reduced, so that every portion of the enamel that protrudes more than a tiny fraction of a millimeter would go out of focus.

In conclusion, analytical procedures depend on the specific needs of the investigation. If the investigation requires detecting the neonatal line for temporal difference between pre- and postnatal trace element acquisition, the method requires the use of a thin section under a microscope. In this case there is no other choice than sectioning and polishing (Lochner et al., 1999; Kang et al., 2004; Dolphin et al., 2005). In other situations, or in case no permission can be granted for sectioning the teeth selected for study, a natural, taphonomic fracture that exposes the inner structure of dental enamel provides a surface that is as good as the one obtained by artificially sectioning the tooth with a diamond-blade saw. In addition, breaking the tooth vertically represents a no-cost alternative to a low speed saw and is preferable to the extent that the tooth can be glued back together without any loss in dental mass, which is unavoidable when teeth are sectioned with a saw. Breaking rather than sawing the teeth also avoids the need for cleaning after sectioning, since there is no contamination of the surface, even though pre-ablating the surface would remove any possible contamination without the need for previous cleaning.

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