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A new method for relative Sr determination in human teeth enamel

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Summary - *We present a new method to determine Sr/Ca changes in hard dental tissues based on laser ablation and spectroscopic detection. By using femtosecond Laser Induced Breakdown Spectroscopy (fs-LIBS), we micro mapped the relative amount of strontium in the enamel of three human lower third molar. We also analyzed the Sr/Ca ratio along the striae of Retzius. Results show that microlibs allows detection of variation in relative Sr/Ca ratio through enamel. The same values of Sr/Ca ratio were found along a single stria. The method has a precision better than 95% and is sensitive enough to detect Sr/Ca ratio variations among striae and within stria. Fs-LIBS generates information in a fast and simple way that can be used by non-specialists to make inferences about diet or mobility in human populations and fossil hominids.*

Keywords - *Laser ablation, Strontium, Calcium, Incremental lines, Enamel.*

Introduction

Strontium/Calcium ratio analysis in bone (e.g. Burton & Wright, 1995) and more recently in enamel has been used to infer paleodiet in fossil hominids (e.g. Elias, 1980; Sillen & Lee-Thorp, 1994; Sillen *et al.*, 1995; Sponheimer *et al.*, 2005). These studies, based on the biological rationale that Sr/Ca ratios are trophic level indicators in a foodweb, have identified different Sr/Ca ratio in distinct hominid species and suggest a possible adaptation in hominin paleodiet (Sponheimer *et al.*, 2005 *contra* Elias, 1980). Sr/Ca ratio in a given species can be used to interpret trophic level if this ratio has already been obtained systematically in plants and mammals living in the same environment. Indeed, geological substrates influence the Sr/Ca ratio at the base of the foodweb changing the relative ratios between groups of plants and mammals. A species with high mobility would present a bone Sr/Ca ratio which reflects all of the substrates the species has passed through during development. Alternatively, it suggests that changes in the diet or mobility can be inferred by modifications in Sr/Ca ratio of the enamel.

Changes in Sr/Ca ratio have been related to periods of growth (Humphrey *et al.*, 2007, 2008a,b). Indeed, two kinds of incremental lines in enamel allow us to obtain chronological information and to establish the pattern of enamel formation. *Striae* of Retzius, the incremental lines with a periodicity that varies from 6 to 11 days among individuals (Fitzgerald, 1998), represent the successive steps of the matrix-forming front of the enamel. These reveal the progression of enamel formation from the first enamel secretion next to the enamel-dentine junction at the cusp tip to the last secreted enamel at cervix. Each *stria* corresponds to a particular moment in time of the enamel formation. Accentuated *striae* of Retzius can be used to match enamel formation periods between different tooth types of the same individual (Reid *et al.,* 1998). A particular accentuated *stria* is the neonatal line which reflects physiological disturbances associated with the birth process. Humphrey *et al.,* (2007, 2008a) have shown that calcium-normalized strontium intensity markedly decreases from birth in infants with exclusive breastfeed; this pattern is clearly seen across the neonatal line in deciduous teeth. Dietary transition from exclusive breastfeeding to complementary food can also be followed through the study of the enamel development (Humphrey *et al.,* 2008b). This suggests that changes in Sr/Ca ratio in the enamel can be related to the incremental lines in enamel and thus that a chronology can be given to changes in Sr/Ca ratio.

Based on this rationale, Sponheimer and colleagues (2005) have analyzed the concentration of Sr in the anterior teeth of robust australopithecines from South Africa and observed that the Sr/Ca ratio differs among perikymata (external manifestation of the *striae* of Retzius). They suggested that these changes reflect the mobility of these early hominids since changes in Sr concentrations reflect different substratum inhabited by these robust australopithecines.

The fact that Sr/Ca ratios can be used to identify modifications in diet or in mobility of individual and populations requires analytical methods sensitive enough to evaluate Sr at trace levels (which means mg/kg, parts per billion). A method which can provide information in a fast and simple way that can be used by non specialists would be the most useful.

There are several analytical methods that allow evaluation the Sr concentration at trace level. The presence of trace elements in teeth can be determined by Inductively Coupled Plasma with detection by Mass Spectroscopy or Optical Emission Spectroscopy (ICP-MS or ICP-OES respectively). Also Atomic Absorption with graphite furnace (Keating *et al.,* 1987) or flame as the atomization device can be used. All those techniques have serious limitations for unique samples studies as fossil teeth. Laser techniques as Laser Ablation coupled with ICP-MS and Laser Induced Breakdown Spectroscopy (LIBS) (Humphrey *et al.,* 2008a; Balter *et al.,* 2008; Miziolek *et al.,* 2006) have noticeable advantages over previous methods. In particular LIBS requires no previous sample preparation, has no limitations in sample dimensions and shapes, requires a low quantity of the sample, with minimal damage, and offers the possibility of in situ analysis. LA-ICP-MS was previously used to measure the distribution of heavy metals (Budd *et al.,* 1998; Grün *et al.,* 2008) and ratios of isotopic carbon in enamel teeth (Sponheimer *et al.,* 2006). LIBS was previously employed in teeth to detect Al (Samek *et al.,* 1999), Mg (Samek *et al.,* 2001; Alvira *et al.,* 2010), Sr (Alvira *et al.,* 2010) and hydroxyapatite (Miziolek *et al.,* 2006).

Femtosecond laser induced Breakdown spectroscopy (femtoLIBS) represents a further improvement. The technique has higher shot to shot stability combined with less sample heating and damage, which makes it an important tool for studying unique pieces that requires preservation. Femtolibs also offers improved spatial resolution that allows more detailed surface mapping. Due to the low quantity of mass ablated by a Femtosecond laser pulse, fs-LIBS can be considered practically almost a non destructive technique (Miziolek *et al.,* 2006; Cremers & Radzienmski 2006; Gurevich & Hergenröder, 2007). Further, it has advantages, with respect to other laser methods, in spatial resolution and depth profiling, that allows obtaining maps distribution and localized analysis.

In this work, we present a new method for determining relative Sr variations in the enamel of human teeth. We use femtoLIBS to micro map the relative amount of strontium in the enamel of three human lower third molar (M3). We also analyzed the Sr/Ca ratio along five *striae* of Retzius.

Materials and Methods

The experimental setup used in this work is shown in Figure 1. It consists in a Ti: Sapphire femtosecond laser system with a CPA (Chirped

Pulse Amplifier). The system delivers laser pulses at = 800 nm, with a duration of τ = 120 fs, an energy of 700 μJ; at a repetition rate of 10 Hz (controlled by an electronic shutter) or in single pulse mode. The energy delivered by the laser was measured by a power meter and controlled by a combination of $\lambda/2$ polarizer and a set of neutral density filters. The laser pulses were focused on the sample by 100 mm focal lens which produce a crater with a diameter of 50 μm. For inspection and monitoring the impact position of the laser on the samples a white light and a CCD camera were used. The samples were mounted on a X-Y-Z translation stage located perpendicular to the direction of laser incidence, this positioning system allows obtaining a sub micrometer lateral resolution.

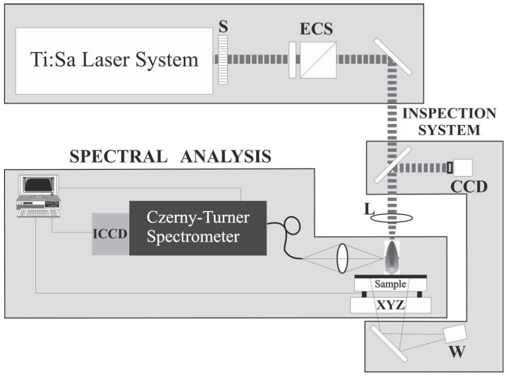

Fig. 1 - Experimental setup. S: Shutter; ECS: Energy Control System; L: focusing lens; XYZ: motorized xyz stages.

The luminescence of the plasma generated after ablation of the surface tooth was collected by a 10 cm focal quartz lens, and focused over a quartz optical fiber. The other side of the fiber was coupled in a 30 cm imaging Czwerny-Turner monochromator (with a 2400 line/mm holographic grating). For detection, an intensified CCD camera (1024x1024 pixels) with time delay and programmable acquisition gate attached to the monochromator was used. This detection system has an optical dispersion of 0.01 nm/pixel and 11 nm range. The delay time of the detector was 2 ns with a gate window of 3 ms the gate window was selected to integrate the plasma emission of at least 20 incident laser pulses. To produce minimal damage on the samples, laser fluences of 30 J/cm^2 were used. This fluence value is slightly up to minimum instrumental detection limit of the system used, and 50 times over the ablation threshold fluence that we measured for enamel (0.6 J/cm^2) which is in agreement with previously reported data (Neev *et al.,* 1996).

Fs-LIBS analysis was performed in three lower M3, one obtained from a medieval archeological site in France (B) (Reid *et al.,* 1998) and two from a modern French population (A1 and A2). Prior to laser analysis, teeth were sectioned across the mesial cusps and lapped down from two sides to obtain sections of approximately 300 microns. Representative lines of Sr (λ=460 nm) and Ca (λ=458 nm) were selected and the distribution of relative strontium traces in the enamel was determined by measuring the calcium-normalized strontium intensity ratio of these lines (this method is known as internal standard) (Miziolek *et al.,* 2006; Singh *et al.,* 2007; Alvira *et al.,* 2010). This treatment and the fact that in our experiments we do not use any external material reference, does not allow us to report absolute concentration. This means that the reported Sr ratios in this paper do not refer to the absolute quantity of this element in the sample. Instead these are relative values that are internally consistent within the sample from which they were extracted. The internal standard method described above prevents problems regarding the stability of the excitation source employed (femtosecond laser in this case). This is because when a relationship of two spectral lines is done, any instrumental factor that could worsen the analytical performance is canceled. In this approximation it is assumed that instrumental factors are not additive but multiplicative.

Figure 2 represents a typical emission spectrum of enamel tooth in the region 450-465 nm showing the emission lines used to obtain the Sr/Ca ratios: 460.73 nm (Sr I) and 458 nm (Ca I), respectively. Sr/Ca ratios were obtained every 500 μm, which allow us to map each tooth with

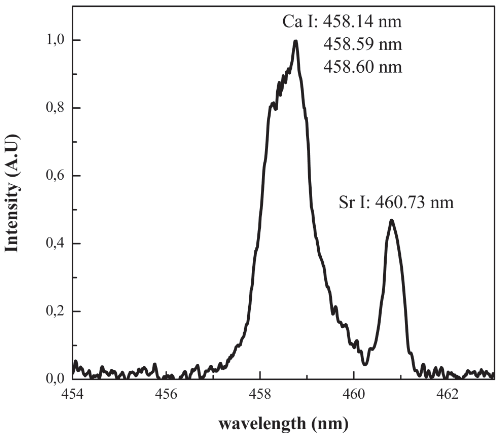

Fig. 2 - Typical emission spectra of enamel tooth in the region 450-465 nm showing the emission lines used to obtain the Sr/Ca ratios. Ca line at λ=458 is the superposition of several lines.

a density surface of 4 shoot/mm^2. Sr/Ca ratios were also measured along incremental lines (*striae* of Retzius) in the enamel of the two modern teeth. Ratio values were determined with a precision above 90% (see discussion).

Results

Distributions maps of calcium-normalized strontium ratio in the three lower M3 analysed are presented in Figure 3. The variation in Sr/Ca ratio differs between teeth; whereas Sr/Ca ratio varies from 0.62 to 0.98 in A2 and from 0.70 to 0.90 in B, variation in A1 is larger ranging from 0.02 to 0.72. In the buccal aspect of A1, the Sr/Ca ratio decreases from the enamel dentine junction (EDJ) to the enamel surface and the highest relative Sr intensity is located on the lateral enamel. In contrast, in the lingual face of this tooth, Sr/Ca ratios fluctuate from cusp to cervix. A noticeable aspect is a high Sr intensity in the middle area between dentine horn and cusp tip in the lingual cusp of this tooth and also in A2.

In A2, relative Sr values become lower from the EDJ to the enamel surface and from cusp tip to cervix. A regular pattern of Sr/Ca ratio distribution is also observed in the medieval tooth but this pattern is different to that observed previously. Three areas can be identified in this tooth, the middle area showing lower values (around 0.75) than the inner (close to the EDJ) and the outer (close to enamel surface) areas where Sr/Ca ratio values are higher (0.85-0.9).

Fs-LIBS analysis was performed following incremental lines in the two modern teeth (Fig. 4). Sr/Ca ratios do not differ significantly along one incremental line or between *striae* from the same tooth. Differences in relative strontium intensity were found between *striae* from different teeth.

Discussion and conclusions

Results show that microlibs allows detection of variations in the relative Sr/Ca ratio through enamel. As shown in Figures 3 and 4 the method is sensitive enough to detect Sr/Ca ratio variations through enamel. In Figure 4b the same values of Sr/Ca ratio were found along each single *stria* within an uncertainly less than 5%. Assuming that the amount of Sr is constant along the *stria*, the precision of the method is better than 95%. On the other hand, if the amount of Sr is not constant along the *stria*, results shown in Figure 4b would express the real fluctuations of the Sr concentration along it. The uncertainty in the Sr/Ca ratio found in Figure 4a is twice than that in Figure 4b. This uncertainty seems not to be due to the method since it was less than 5% in the other tooth. Thus, different results along a single *stria* of Retzius can effectively reflect the variation along the incremental line. We estimate that the method has the sensitivity to detect changes in Sr/Ca intensities of less than 5%.

The close values of Sr/Ca ratios along the same *striae* indicate very similar relative strontium intensity at a given moment. However, fs-LIBs analysis along *striae* was performed in areas which show very low variation in Sr/Ca ratios as it is revealed by enamel maps (Fig. 3). Indeed, the *striae* followed are located in the central third of the cuspal area, a region where the relative amount of strontium shows low variation.

There are two main types of age related changes that could cause a shift in the strontium signal in enamel - a change in diet or a change in location. Previous studies have looked at the relationship between these changes and the individual chronology, mainly based on the study of incremental lines in enamel. Indeed, significant changes in calcium-normalized strontium ratio across the neonatal line have been interpreted in relation to a shift at birth to a particular diet (exclusive breastfeeding) and suggest a possible chronological distribution of Sr/Ca ratio changes in the enamel of deciduous teeth (Humphrey *et al.,* 2007, 2008a,b). Our analysis of relative strontium intensity of lower M3s would enable us to follow changes which have occurred from 9 to 13 years of age since the crown of M3 is formed during this period of growth (Liversidge, 2008). If Sr/Ca ratio variations follow a chronological pattern, it would be expected that changes in Sr/Ca ratio would reflect the pattern of incremental lines in enamel. However, the objective of this paper was not to identify whether there is a chronological signal in human tooth enamel since we lack the basic information about these individuals required to carry out such kind of study. More precisely the mapping of Sr/Ca ratios reveals a pattern of Sr/Ca ratio distribution that does not reflect the pattern of incremental lines. The mapping of Sr/Ca ratios within these M3s reveals a complex distribution of Sr/Ca ratios throughout enamel. We have no information about changes or absence of changes in locality and diet for these three individuals. One possibility would be that there is no correlation between the distribution of Sr/Ca ratios and enamel formation chronology, but most likely the three individuals used in the study did not migrate or change diet between the ages of 9 and 13 years. If that was the case, then we are seeing the background noise in a tooth formed during a period of no change in diet and/or locality during this period of growth.

In conclusion, we present a new method to determine Sr/Ca changes in hard dental tissues based on laser ablation and spectroscopic detection. By using femtosecond Laser Induced

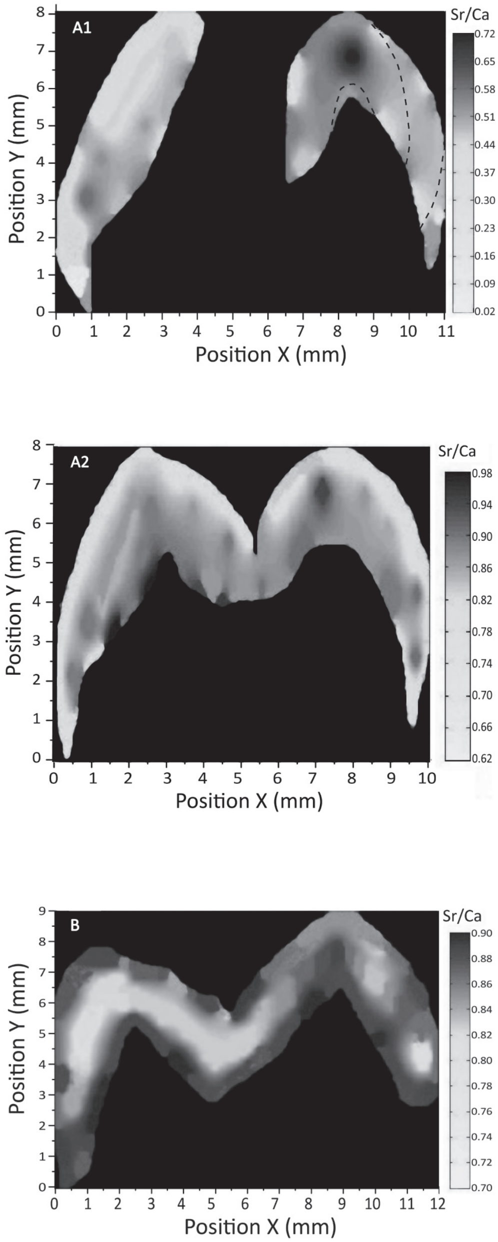

Fig. 3 - Maps of Sr/Ca ratios distribution in lower M3s. A1, A2 are modern teeth. B is a medieval tooth. The buccal face is on the left and lingual face on the right. Dashed lines (A1) show disposition of incremental lines (striae of Retzius) in enamel.

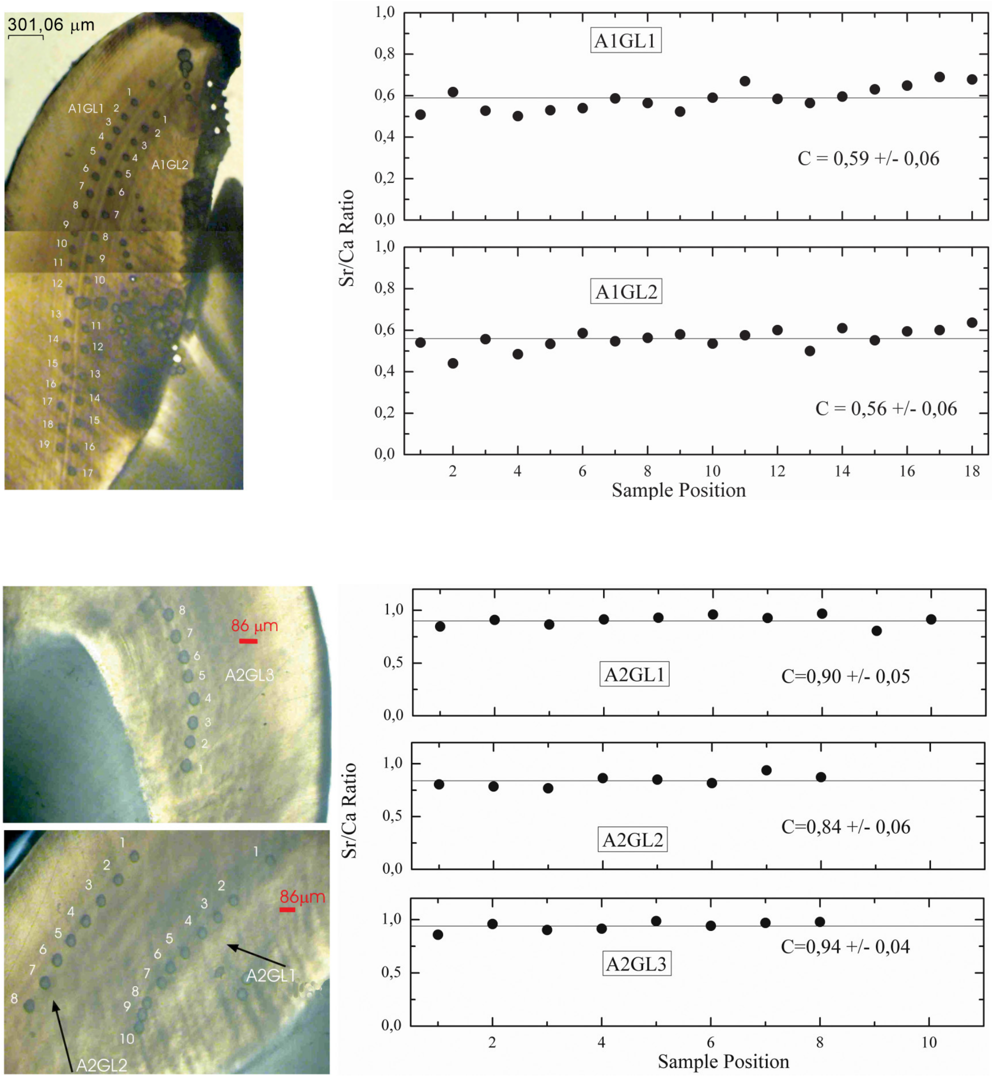

Fig. 4 - a) Laser dots along two growing lines in the buccal aspect of A1 lower M3. b) Laser dots along two growing lines in the buccal aspect and one growing line in the lingual aspect of A2 lower M3. Numbers in dots refer to Sr/Ca ratios shown in the plot on the right. It is worth noting that incremental lines were followed in areas in which maps revealed that Sr/Ca ratios are more or less stable.

Breakdown Spectroscopy we show that it is possible to obtain this information in a fast and simple way that can be used by non specialist to make inferences about diet or mobility in human populations and fossil hominids.

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