Buccal dental microwear and stable isotopes of El Collado: A mesolithic site from Spain

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Abstract

This study examines the correlation between buccal dental microwear and stable isotopes. The buccal surface of post-canine teeth casts from El Collado, the largest Mesolithic site in Spain, were examined under Scanning Electron Microscope; photomicrographs were taken from the middle third of the buccal surface with magnification 100X. Only six individuals passed the criteria for buccal dental microwear analysis. The photomicrographs were treated by adobe Photoshop 8.01 to cover an area 0.56 mm^2 of middle third of buccal surface, the output photomicrographs were digitized using Sigmascan Pro 5 by SPSS. Then the correlation between buccal microwear pattern and stable isotopes of the same individuals, of the previous study of Guixe et al., 2006, was examined using a Pearson test. Statistical analysis revealed that there is no significant correlation between stable isotopes and buccal dental microwear of the people of the Mesolithic site of El Collado. The historical and archaeological documentation suggest that the Mesolithic people tended to consume marine food. Fish-drying techniques were used during the Mesolithic period which allowed the introduction of dust and sand to the fish. These abrasive particles affected the buccal dental microwear pattern, so that no correlation between the isotopes and microwear may be expected.

This also suggests that the buccal dental microwear pattern exceeds dietary reconstruction to reconstruct food processing techniques.

Keywords: Microwear; El Collado; Diet

Introduction

Diet is a fundamental aspect in understanding culture, paleobiology and the evolutionary history of ancient human populations and hominin species. Reconstructing paleodiet from the archaeological sites is usually dependant on plants and animal remains. But the presence of these remains does not mean that it was an important part of the diet of the ancient population. Therefore, bioarchaeologists and paleontologists tend to extract dietary information using direct methods or techniques which focus on the skeletal remains.

One technique has been the use of chemical analysis of bone and teeth. Bone chemistry provides strong documentation of the diet of past human populations. The most important kind of chemical analysis used in dietary and nutritional studies is measuring the ratios of certain stable isotopes in skeletal and dental tissues of ancient human populations. Most studies used stable carbon isotopes 13C and 12C. The ratio of carbon stable isotopes in humans is dependent on the plants that were eaten by human during life.

Human who consume terrestrial plants and animal proteins have δ 15N values in bone collagen of about 6–10 ‰ whereas consumers of marine food sources may have δ 15N values of 15–20 ‰ (1). Humans that consume only terrestrial protein sources may have collagen δ13C values of about -20‰, while humans that consume the majority (>90%) of their protein from marine sources may have collagen δ13C values close to -12‰ (2).

A widely used technique to reconstruct diet is dental microwear analysisMicrowear appears as microscopic features (pits and striations) on the occlusal surface of a tooth, caused by abrasive particles and tooth to tooth contact (4, 31). The presence of these features on the buccal surfaces of teeth is caused by abrasive particles in the diet (5-10). The microwear pattern is determined according to the number of striations, length and orientation on the buccal surfaces, which have been proven to be correlated with dietary hardness and abrasiveness (6, 9, 11-13).The hard texture of diet usually comes from two sources: the abrasive particles from the opal phytoliths of the plant tissue (14,15), or the contamination of food by sand and dust during its processing or in the natural environment (8, 12,16).

The length, orientation and the number of striations on the buccal surface vary according to the food consumed by an individual. Meat based diets tend to create more vertical striations compared to plant based diets which tend to form more horizontal striations (9, 11, 17-19). However, harder foodstuffs, due the presence of abrasive particles, tend to generate more striations (7, 8, 20).

The total number of striations or the density of striations is the most indicative variables for dietary reconstruction. The higher density of striations in Neanderthal teeth is due to more plant consumption (7). The higher density of buccal microwear striations in H. heidelbergensis teeth from Sima de los Huesos was due to more abrasive dietary habits (6). In addition, the higher density of striations in the Mesolithic population from Portugal is due to contamination during food processing (8). Also the length of buccal microwear striations is highly correlated with the texture of diets that require more masticatory demand. The very long striations on the teeth of Neolithic populations of the Near East are due the introduction of cereals in diet, accompanied by the use of grinding stones (21). The index of the length of horizontal and vertical striations can be used to classify human populations depending on diet to: pastoralists, agriculturists, carnivores, and hunter-gathers (5, 9, 11).The first evidence of extensive marine food intake was reported from the costal Mesolithic populations in Atlantic areas of Europe (22, 23) and the Baltic (24). While inland the people of the Mesolithic were less dependent on marine food than plant consumption, which was assessed by stable Isotopes and buccal dental microwear analyses (21, 25).

These two techniques (stable isotopes and buccal microwear) have been common in biological anthropology, but unfortunately the correlation between both of them has not been considered. Accordingly, this study tests the correlation between stable isotopes of dental enamel and the number of buccal microwear striations.

The site

El Collado is the largest Mesolithic site in the Spanish Levant, this site is located near the city of Oliva (Valencia), about 3 km from the eastern cost of Spain (Figure 1). The area of the site has a rich ecosystem which allowed continuous occupation from middle Paleolithic to the Neolithic and later periods as well (26). Fifteen graves were excavated from the site and radiocarbon dated to 7,57±160 BP and 7,640±120 BP (26,27), which was calibrated to 6630–6250BC (28,29).

Materials and Methods

Since the buccal microwear pattern is an accumulative process that depends on the age of an individual and time since tooth eruption (5, 12 ,21), only adult individuals was sampled for this study. The samples are characterized by being well-preserved without dental pathologies. The sampled teeth represent six adult individuals. Before examining the teeth under Scanning Electron Microscope, the teeth were gently cleaned with pure acetone, and then rinsed with ethanol (70%) to eliminate any grits that may mask microwear features. Molds of the original teeth were obtained using Polyvinyl-siloxane President Microsystems™ (Coltene Regular Body) (30,31), and the positive casts were obtained using epoxy resin (Epo-Tek 301, By QdA) with a two-stage centrifugation to prevent the formation of air bubbles that may mask the striations on the buccal surface.

Then the casts were mounted on aluminum stubs using thermo-fusible gum, the gums were then stained using a colloidal silver stain. The casts were then coated with a 400 Å gold layer. The SEM settings were 15 KV with 0º tilt angle of the secondary electrons to meet the standard of the buccal microwear technique. All the micrographs were obtained at 100X magnification, covering an exact area of 0.56 mm2 of enamel on the medial third of the buccal side of the tooth crown. The side of each square image measures 748.331 µm. Each image was processed by a high pass (50 pixel) filter and automatic level enhancement using Photoshop (Adobe CS 8.2.1), Figure 2. The measurements of striations were made using a special program SigmaScan Pro5 by SPSS. The microwear pattern of each analyzed micrograph included the number (N), average length (X) and standard deviation of the length (S) of all observed striations (T), the striations were divided by categories of orientation in 45-degree intervals: vertical (V), horizontal (H), mesio-occlusal to disto-cervical (MD), and disto-occlusal to mesiocervical (DM). Thus, a total of 15 variables (N, X and S for T, V, H, MD and DM orientations) were derived for the sample studied. All the variables studied passed Kolmogorov-Smirnov Normality tests.

The data of stable isotopes were obtained from a previous study considering the same individuals of this study (25). Using SPSS 15, Pearson correlation was performed between all the variables of buccal microwear pattern and isotopes results (δ 13C, δ 15N, %C, and %N).

Results and Discussion

The descriptive statistics of buccal microwear pattern and stable isotopes of each individual are shown in Table 1. Kolmogorov-Smirnov normality tests showed that none of buccal microwear variables differed significantly from normality. Therefore, the parametric statistical tests could be applied to the raw buccal microwear data No statistically significant correlations were discovered between buccal microwear and stable isotopes using Pearson correlation.

Stable isotope analysis is widely used as an informative tool in dietary reconstruction. But this technique is affected by many factors that could modify the results; among them is digenesis (32-35).

Stable isotopes or trace elements could not reflect the texture of the food, but they reflect the chemical constitute of food (36-40). Unfortunately, food processing can affect both the chemical analysis of teeth by changing the chemical constitute of bone through adding elements and compounds from food utensils to teeth (46)

During the Mesolithic period, the subsistence economy was dependent on marine resources (23, 25, 41). It is known that ancient human populations tended to use fish-drying techniques as a tool of food processing (8, 42-44). This type of food processing increased the chance of contamination with dust which could affect the tooth structure. This phenomenon was noticed in ancient population of the Arabian Gulf, who exhibited a high rate of dental attrition (42,43), and the same results were found in Eskimo samples (44). When comparing the buccal dental microwear of the people of the Mesolithic of Portugal and the Natufians of the Near East, the Mesolithic samples show high tendency of buccal microwear features probably due to the contamination of their diet (fish) (8).

It is known that using utensils in food processing came after the introduction of pottery during the Neolithic period (45). Accordingly, chemicals from utensils did not affect the chemistry of teeth in the study. Therefore, the lack of correlation between stable isotopes and buccal dental microwear does not come from stable isotopes.

Food processing through fish-drying could affect the hardness of the diet by introducing more abrasive materials which cause microwear patterns, but not stable isotopes. The introduction of the abrasive particles during food processing, in the form of dust entering from the environment, may be the reason forhigher dental microwear densities than usual, and a lack of correlation between buccal dental microwear and stable isotopes.

Food processing techniques are important issues used to reconstruct the past life of human populations. This issue can not be detected by stable isotopes alone, but buccal microwear can be used to reconstruct food processing techniques as well as diet.

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Figure 1. The Location of El Collado (25)

Ind. No.	δ ^{13}C (%0)	δ ^{15}N (%0)	C:N	%C	%N	NH	XH	SH	NV	XV
1	-19.500	10.200	3.400	23.700	8.200	37.000	75.238	49.502	67.000	84.277
2	-19.100	8.900	3.300	24.900	8.900	29.000	69.010	38.339	11.000	126.717
3	- 17.600	10.200	3.200	15.900	5.800	35.000	171.647	166.155	5.000	160.329
4	-17.600	12.800	3.400	24.100	8.300	42.000	86.367	56.698	27.000	90.787
5	-18.200	10.600	3.300	21.700	7.600	20.000	268.911	219.548	10.000	82.990
6	-18.200	10.900	3.300	27.000	9.500	25.000	83.236	108.868	4.000	83.293
Ind. No.	**SV**	**NMD**	**XMD**	**SMD**	**NDM**	**XDM**	**SDM**	**NT**	**XT**	**ST**
1	51.741	114.000	78.258	68.311	14.000	78.404	61.321	232.000	79.523	60.427
2	112.124	51.000	66.482	59.065	37.000	71.664	45.395	128.000	73.729	59.517
3	72.967	22.000	76.474	44.172	31.000	98.516	136.219	93.000	124.147	136.472
4	55.552	114.000	69.592	61.953	24.000	60.490	24.669	207.000	74.705	57.465
5	61.762	24.000	120.716	79.300	22.000	121.053	111.527	76.000	154.848	151.478
6	62.418	26.000	82.922	64.059	21.000	135.970	110.946	76.000	97.703	95.736

Table 1. Descriptive statistics of microwear and stable isotopes

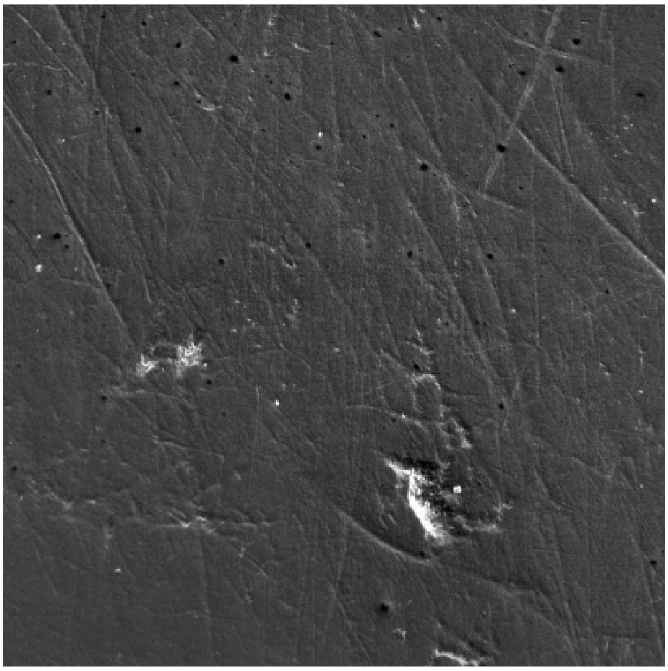

Figure 2. Cropped micrograph