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Analysis of dental collagen cross-linking and carbonate mineral integration from Roman/Byzantine sites in Jordan using ATR-FTIR spectroscopy*

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Abstract

Existing bioarchaeological studies have not fully explored the analytical characterization of dentine's chemical structure. Accordingly, this study used ATR-FTIR to analyze the dentine of 30 human teeth from six archaeological sites in Jordan dating from the Early Roman to the Early Byzantine periods. In addition to collagen cross-linking, we analyzed the factors (ratios) of phosphate/amide I, carbonate/phosphate, and A-type carbonate/B-type carbonate that may influence dentine strength. The results of the study point to statistically insignificant differences in the factors affecting the tensile strength of dentine and thus tooth loading at the site level, while a statistically significant one is found among sites. The differences in these ratios are found to be attributed to tooth loading and age at death.

Keywords: dental collagen; ATR-FTIR spectroscopy; Jordan

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Introduction

Teeth are the most surviving human remains at archaeological sites, and fortunately, they lock inside tremendous amounts of information that bioarchaeologists have utilized to reconstruct the people's past. Dental tissues have been extensively examined, especially dentine, where, unlike enamel, it holds data pertaining to an individual's entire life. Recently, researchers have been looking at archaeological dentine to reconstruct diet and physiologic stress (1-6), to determine age (7-9), to find out dentine metabolites (10, 11), to date skeletal remains (12), and to find out how cortisol levels indicate stress (13, 14). However, non-bioarchaeologists have done a lot of research on dentine (15-26). No bioarchaeologists have investigated its chemical structure and characterization.

There was a need for more knowledge on the dentine matrix and its structural changes in response to intrinsic (physiological and pathological) and extrinsic (masticatory forces). The chemical structure of dentine is not static but continuously changes in response to diet, tooth loading, and age (27). Precise knowledge of these chemical changes advances our understanding and interpretation of past people's lifeways and environments. In this regard, the less destructive analytical technique is ATR-FTIR (28, 29), where bioarchaeologists have used this technique mainly to assess diagenetic contamination due to the loss of crystalline structure (30, 31).

Dentine is secreted by odontoblasts and consists of mineral hydroxyapatite (70%), collagen (20%), and water (10%) (32). The collagen forms a densely mineralized collagenous matrix, enclosing the detal pulp and providing attachment for the enamel and cementum (33). It has three major types: primary dentine, which forms during tooth formation and represents the bulk of the tooth; secondary dentine, which starts forming after crown completion; and tertiary dentine, which lines up the pulp and has a defensive role in pulp protection (27). The tertiary dentine is formed in response to external trauma, attrition, abrasion, erosion, and caries (34, 35). Masticatory forces and/or tooth loading affect the collagen structure of all dentine types over time, but collagen cross-linking prevents degradation (36, 37). Collagen cross-linking occurs in dentine to provide tensile strength and viscoelasticity in response to mechanical, physiological, and pathological stresses (38-40). In addition, collagen cross-linking may occur as a response to aging (41), with the formation of sclerotic dentine (more mineralized dentine) and dead tracts (42). Dental sclerosis starts at a late teen age near the apex of the root, which appears transparent under a microscope (43).

Collagen cross-linking varies by tooth type, which is greater in molars (44). The collagen cross-linking that has been investigated spectroscopically are pyridinoline and divalent cross-links (18, 45), which is determined through the quantification of the amide I peak at ~1,650 cm^{-1} (46). The ratio of the amid I sub bands of ~1,660 cm^{-1} (nonreducible collagen: pyridinoline) to ~1,690 cm^{-1} (reducible collagen: dehydrodihydroxylysinonorleucine [DHLNL]) is usually calculated to quantify collagen denaturation (18). In denaturized or younger collagen, the relative intensity ~1,660 cm^{-1} decreases while ~1,690 cm^{-1} increases (41, 47). The other changes that may take place in collagen (caused by either intrinsic or extrinsic factors) include the incorporation of carbonates in the mineral phosphate, leading to a less stable or deformed crystal lattice within the collagen (21). The incorporated carbonates are either A-type or B-type, located at 879 and 872 cm^{-1} respectively (48). In addition, the phosphate to amide I ratio may also be altered due to the above factors and can be evaluated as the ratio between 1,035 and 1,655 cm^{-1} (49). To understand the collagen cross-linking of pyridinoline and DHLNL, the mineral matrix ratio, and the carbonate mineral ratio in archaeological dentine, this study uses ATR-FTIR spectroscopic analyses on the dentine of 30 teeth from six archaeological sites in Jordan: Abila, Natfeh, Sa'ad, Udhruh, Ya'mun, and Yasieleh. Based on the previous literature, the study seeks to test two null hypotheses:

H01: The means of the ratios [A1,035/A1,655, $A_{872}/A_{1,035}$, $I_{1,660}/I_{1,690}$, and I_{897}/I_{872}] grouped by a site are the same. In other words, each site displays no differences in the mean values of these ratios among its individuals (no. = 5 for each site).

H_{02}: There are no interactions among the sites. In other words, any ratio remains the same despite which site it belongs to (no. = 30).

Materials and methods

The study comprises 30 teeth from six archaeological sites in Jordan (5 teeth each) dated to the periods of Early Roman (BC 63 – AD 135), Late Roman (135 - AD 324), and Early Byzantine (324 – AD 491). The sites are Abila, Natfeh, Sa'ad, Udhruh, Ya'mun, and Yasieleh. All of them are located in northern Jordan, except for Udhruh in the south (Fig. 1). All the sampled teeth

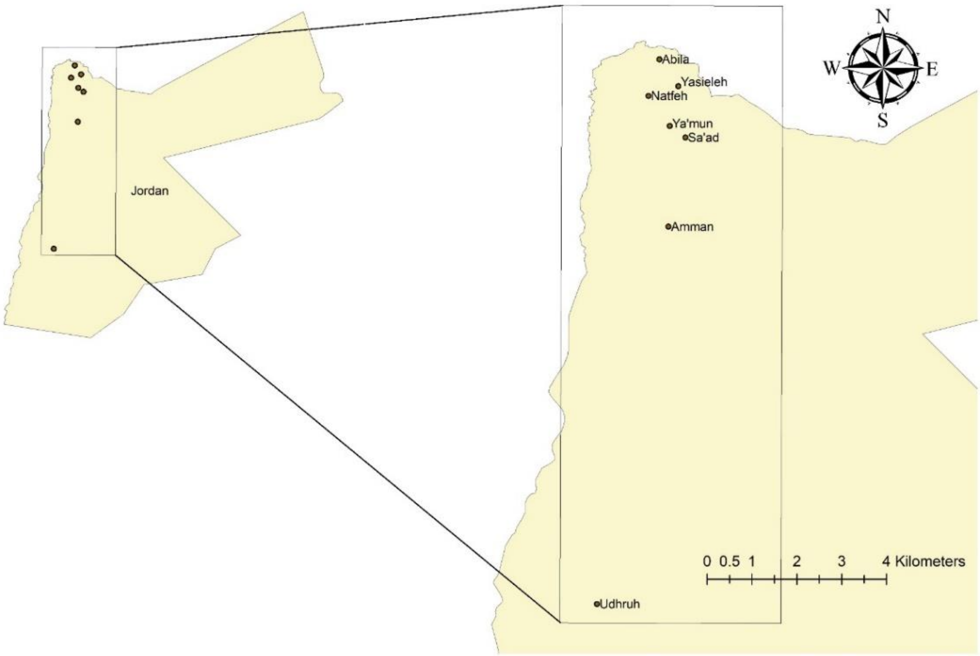

Figure 1. The location of the sampled archaeological sites.

are permanent, loose (not in occlusion), and in good preservation conditions. The sampling procedure ensured that each tooth belonged to a different individual based on side identification (left and right) and locus number. All of the sampled teeth were free of carious lesions, and of minimal dental wear (dentine is not exposed). Sexing and aging of the teeth could not be established as the teeth were not in occlusion. Third molars were excluded during sampling because they are the last to erupt and the least affected by tooth loading. The descriptions of the samples are shown in Table 1 and include site name, sample no., tomb no., date, and tooth type. Sample preparation and analysis were performed at the Departments of Anthropology and Chemistry at East Carolina University. The samples were cleaned manually to remove the surface dirt using a surgical blade, then ultrasonically in a water bath, and let dry overnight. Each tooth was embedded in epoxy overnight and cut vertically using a diamond saw at a low speed. The surface of the section was polished to a smooth and even surface. The ATR-FTIR spectra were collected using the Nicolet iS50FT-IR unit equipped with ATR diamond crabapple of scanning an area of less than 10 microns. The scanning used an absorbance mode of 16 scans at a resolution of 4 cm^{-1} and a range of 400-400 cm^{-1}. An atmospheric correction was applied to remove the contribution of the atmospheric background. The spectrum of each tooth (from 800 - 1750 cm^{-1}) was analyzed separately after baseline and ATR corrections. Fourier self-deconvolution was then performed to enhance the resolution and extract more information from overlapping peaks in a spectrum (50).

The mineral-to-matrix ratio is calculated by the band ratio of $A_{1,035}/A_{1,655}$ (49). This ratio corresponds to the vibrations of the hydroxyapatite phosphate ion (v_3) and the collagen amide I (C=O stretching) (20). The band ratio of $I_{1,660}/I_{1,690}$ was also calculated to measure the amount of nonreducible to reducible cross-links in collagen (19). The distribution of carbonate within the mineral matrix of collagen was calculated by the band ratio of $A_{872}/A_{1,035}$ (20). The relative content of A-type and B-type carbonate was calculated by the band ratio of I_{879}/I_{872} (51). The calculations of the length of the peaks and the area under the peaks were

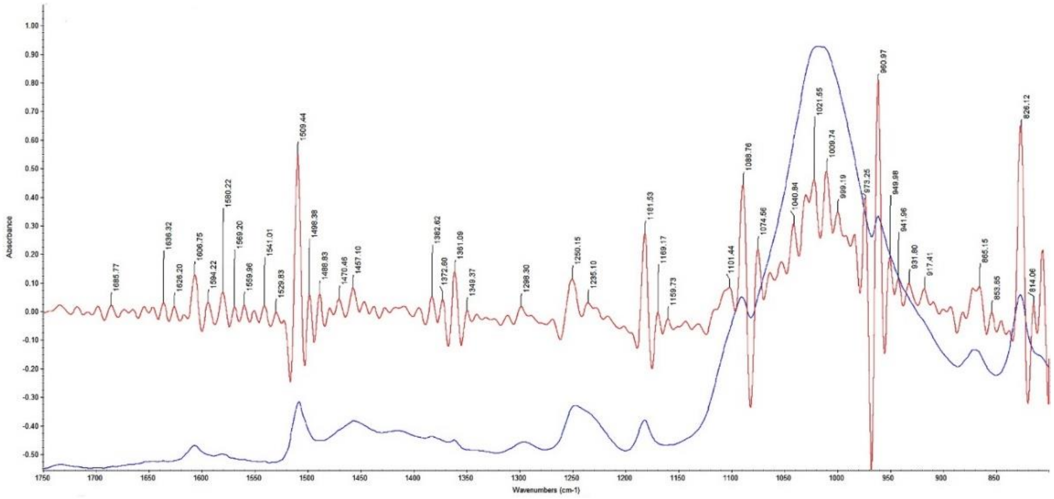

Figure 2. Dentine spectra in blue with Fourier Self-Deconvolution in red: a sample from Abila.

performed using Omnic 9.2 software. The resulting values were analyzed using two-factor analysis of variance with replication (ANOVA) in the Excel program.

Results

The hypotheses of the study were tested using a two-factor ANOVA with replication. There is evidence that the first null hypothesis (H01) is true because the means of the ratios [$A_{1,035}/A_{1,655}$, $A_{872}/A_{1,035}$, $I_{1,660}/I_{1,690}$, and I_{897}/I_{872}] grouped by site are the same. The P-value of 0.11 is higher than the expected value of $\alpha = 0.05$, and the value of F (1.83) is lower than the value of F critical (2.30) (Table 3). These results indicate higher homogeneity of samples at the site level.

The second null hypothesis (H_{02}: There are no interactions among the sites. In other words, any ratio that remains the same despite which site it belongs to) is rejected as the P value (0.004) is less than $\alpha = 0.05$ and F (2.47) > F critical (1.77). In other words, there are interactions among the six sites.

The phosphate to amide I ratios ($A_{1,035}/A_{1,655}$) are higher in Abila and Ya'mun, implying a relatively higher mineralization of dentine, while the values of phosphate in the other sites did not exceed the values of amide I. The sites of Udhruh showed the highest incorporation of carbonate in the crystal lattice of apatite, as indicated by the ratio of $A_{872}/A_{1,035}$ followed by Sa'ad, Yasieleh, Ya'mun, Natfeh, and Abila. The ratio of nonreducible to reducible collagen cross-links indicated by $I_{1,660}/I_{1,690}$ also varied among the six sites, where the highest mean value was recorded for Ya'mun and Abila. Carbonate incorporation may occur at the PO_4 site (B-type carbonate: I_{872}) or the OH site (A-type carbonate: I_{879}) (48). In the samples of Ya'mun, Sa'ad, and Yasieleh, the substitution occurred more at the site of OH in hydroxyapatite, while in Abila, Udhruh, and Natfeh, the substitution occurred more at the PO_4 site in hydroxyapatite.

Discussion

The ANOVA statistics failed to reject the first null hypothesis that the means of the ratios [$A_{1,035}/A_{1,655}$, $A_{872}/A_{1,035}$, $I_{1,660}/I_{1,690}$, and I_{897}/I_{872}] grouped by a site are the same. This implies the presence of inter site consistency or a homogenous sample at each site. The differences appeared when comparing the sites with their ratios among each other's, which proved the rejection of the second null hypothesis, which states that there are no interactions among the sites. These differences were attributed to different factors, as explained below, however, the attributed factors varied across time as the samples span a period of about 550 years.

The higher phosphate-to-amide I ratio ($A_{1,035}/A_{1,655}$) in Abila and Ya'mun is best explained as an artefact of tooth maturation. The sampled teeth from these sites probably belong to relatively older individuals. Previous studies showed that the higher ratio of $A_{1,035}/A_{1,655}$ in dentine is attributed to carious lesions and aging (52, 53). However, the sampled teeth in this study were caries-free, so aging stands as a proper explanation. In this regard, many previous studies estimated the average age at death in

Table 1. The samples of the study.

Site	No.	Sample no.	Tomb	Date	Tooth type
Sa'ad	1	2956	Cave 2B	LR	RM^1
	2	1000	1	EB	RM_3
	3	1001	1	EB	RM_2
	4	1002	1	EB	RM_1
	5	1003	1	EB	RM_1
Ya'mun	6	200	198	LR	RM_2
	7	202	198	LR	RM_2
	8	205	45	LR	RM_1
	9	206	45	LR	RM_1
	10	359	45	LR	RM_1
Yasieleh	11	50	361	LB	LM_1
	12	57	361	LB	LM_1
	13	76	361	LB	LM_1
	14	77	361	LB	LM_1
	15	78	361	LB	LM_1
Natfeh	16	400	21	ER	LM^1
	17	402	21	ER	LM^2
	18	403	21	ER	LM^2
	19	404	21	ER	LM^2
	20	405	21	ER	LM^2
Abila	21	300	82	EB	RC^u
	22	301	82	EB	RC^u
	23	302	82	EB	LC^u
	24	303	82	EB	RC^u
	25	304	82	EB	LC^u
Udhruh	26	103	Square 6	LR	RM_2
	27	104	Square 6	LR	RM_2
	28	105	Square 6	LR	RM_2
	29	108	Square 6	LR	RM_2
	30	110	Square 6	LR	RM_2

Table 2. The average ratios of $A_{1,035}/A_{1,655}$, $A_{872}/A_{1,035}$, and $I_{1,660}/I_{1,690}$ of the six sites.

Site	$A_{1,035}/A_{1,655}$	$A_{872}/A_{1,035}$	$I_{1,660}/I_{1,690}$	I_{879}/I_{872}
Abila	1.29	2.47	2.30	0.81
Natfeh	0.35	4.05	0.55	0.63
Sa'ad	0.60	8.95	0.86	1.00
Udhruh	0.34	19.53	0.96	0.73
Ya'mun	1.09	4.99	2.47	3.84
Yasieleh	0.37	6.96	0.91	1.83

Table 3. Results of ANOVA.

Source of Variation	SS	df	MS	F	P-value	F crit
Sample	200.47	5	40.09	1.83	0.11	2.30
Interaction	809.17	15	53.94	2.47	0.004	1.77
Within	2096.06	96	21.83			
Total	4116.10	119				

Abila and Ya'mun to be between 22 and 35 years, owing to several environmental factors including infectious diseases, earthquakes, and maternal complications (54-59).

The incorporation of carbonate into the mineral matrix of collagen changes both the physical structure and chemical stability of the apatite crystals (21). Udhruh showed the highest incorporation of carbonate in the crystal lattice of apatite ($A_{872}/A_{1,035}$), followed by Sa'ad, Yasieleh, Ya'mun, Natfeh, and Abila. However, less carbonate is usually deposited in sclerotized or aged dentine (20). This relationship is seen clearly in the data, where the correlation coefficient between $A_{872}/A_{1,035}$ and $A_{1,035}/A_{1,655}$ among all sites is -0.38. The correlation coefficient increases within a site; for example, the correlation coefficients for Abila, Natfeh, Sa'ad, Udhruh, Ya'mun, and Yasieleh are -0.95, -0.74, -0.6, -0.55, and -0.33, respectively. These results are indirect indications of age variations among the selected samples.

The carbonate is added through A- or B-type substitutions, which cause the a-axis in the apatite lattice to grow or shrink (60). The B-type carbonate increases with age (61-63), leading to a smaller ratio of I_{897}/I_{872} as in Natfeh and Udhruh, which is in congruency with the deducted age from the ratio of $A_{1,035}/A_{1,655}$ for Ya'mun as being older.

The differences in the ratio of $I_{1,660}/I_{1,690}$ among the sites imply that they had different tooth loadings during life (41, 47). The ratios of $I_{1,660}/I_{1,690}$ in Abila and Ya'mun are 2.3 and 2.47, which are higher than the reported ratios from the other sites, which indicates more tooth loading. According to Sandias and Müldner (64), the human diet at Ya'mun was predominately based on terrestrial resources, mostly legumes, with little dietary variability. Such food consumption requires higher masticatory forces (65). The excavated teeth from Abila showed heavy wear and consequently hard food consumption, as well as higher masticatory forces (66).

The dental loadings across the six sites are notably uneven, likely attributed to the diverse dietary practices prevalent at each one (67-71). The period spanning from BC 63 to AD 491 witnessed the presence of a flourishing population characterized by a wide spectrum of food qualities and probably mobility, leading to wider tooth loadings (72). It's substantial that not all sites had equal access to technological innovations that facilitated food acquisition and processing (73). An example on tooth loading was represented by the depth of the temporomandibular joints among the inhabitants of the Late Roman site of QAIA and the Early Byzantine site of Wadi Faynan (74). Their findings pointed out that the Wadi Faynan site exhibited harder food consumption, resulting in a higher degree of tooth loading compared to the Late Roman site of QAIA. This variation in tooth loading emphasizes the nuanced influence of food accessibility and technological advancements on oral health within these archaeological contexts.

Conclusions

The detailed structure of collagen in human dentine from archaeological teeth and how collagen cross-linking changes in response to tooth loading and aging have remained barely touched on in the bioarchaeological lexicon. The use of ATR-FTIR on the archaeological dentine demonstrates the ability to unravel these changes and pinpoint the cause of change in collagen cross-linking. The study tracked the collagen structure in dentine over a period spanning about 550 years. The results indicate considerable changes, which were attributed to the varied tooth loadings of the sampled individuals.

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Declaration of Interest

None

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