The dawn of dentistry in the late upper Paleolithic: An early case of pathological intervention at Riparo Fredian

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ABSTRACT

Objectives: Early evidence for the treatment of dental pathology is found primarily among food-producing societies associated with high levels of oral pathology. However, some Late Pleistocene hunter-gatherers show extensive oral pathology, suggesting that experimentation with therapeutic dental interventions may have greater antiquity. Here we report the second earliest probable evidence for dentistry in a Late Upper Paleolithic hunter-gatherer recovered from Riparo Fredian (Tuscany, Italy).

Materials and Methods: The Fredian 5 human consists of an associated maxillary anterior dentition with antemortem exposure of both upper first incisor (I^1) pulp chambers. The pulp chambers present probable antemortem modifications that warrant in-depth analyses and direct dating. Scanning electron microscopy (SEM), microCT and residue analyses were used to investigate the purported modifications of external and internal surfaces of each I^1.

Results: The direct date places Fredian 5 between 13,000-12,740 calendar years ago. Both pulp chambers were circumferentially enlarged prior to the death of this individual. Occlusal dentine flaking on the margin of the cavities and striations on their internal aspects suggest anthropic manipulation. Residue analyses revealed a conglomerate of bitumen, vegetal fibers, and probable hairs adherent to the internal walls of the cavities.

Discussion: The results are consistent with tool-assisted manipulation to remove necrotic or infected pulp *in vivo* and the subsequent use of a composite, organic filling. Fredian 5 confirms the practice of dentistry – specifically, a pathology-induced intervention – among Late Pleistocene hunter-gatherers. As such, it appears that fundamental perceptions of biomedical knowledge and practice were in place long before the socioeconomic changes associated with the transition to food production in the Neolithic.

START MANUSCRIPT

To date, the earliest examples of definitive prehistoric dentistry come from Neolithic contexts. A Neolithic graveyard (MR3) at Mehrgarh in Pakistan contained 11 drilled teeth, belonging to nine individuals, of which at least four of the teeth had associated decay (Coppa et al., 2006). It is not possible determinate whether the lack of decay in the remaining seven teeth was due to successful removal of infected dental tissue. An individual from a Danish Neolithic passage grave at Hulbjerg exhibits drilling near the bifurcation of the right M^2 roots (Bennike and Alexandersen, 2003; Bennike and Fredebo, 1986). The individual also exhibits periodontal disease and caries suggesting that drilling was related to pathological intervention (Bennike and Alexandersen, 2003). A final example of an early dental intervention concerns a 'beeswax' filling from Neolithic Slovenia, which was probably used to seal an antemortem/perimortem crown fracture for palliative purposes (Bernardini et al., 2012). While many more chronologically-recent cases of pathology-induced dental interventions are well-documented among both food-producers and hunter-gatherers from Old and New World contexts (Bennike and Alexandersen, 2003; Ortiz et al., 2016, Schwartz et al., 1995; Seidel et al., 2005; Turner, 2004, White et al., 1997), there is little evidence for similar pathological interventions preceding the Neolithic.

An exception is a Late Upper Paleolithic specimen from Villabruna (Sovramonte – Belluno, Italy, directly dated to 14,160-13,820 calendar years ago [cal BP]) (Vercellotti et al., 2008). The Villabruna 1 individual exhibits caries on the right M_3 that was clearly manipulated with a lithic tool *in vivo* in an effort to partially clean decay through scraping and levering actions (Oxilia et al., 2015). However, the location of the caries in the distal-most portion of the mouth would have made it very difficult to fully clean and may explain why this manipulation was less extensive than many of the more obvious drilling interventions in later prehistoric and historic examples (e.g. Bennike and Alexandersen, 2003; Coppa et al., 2006; Ortiz et al., 2016; Schwartz et al., 1995; Seidel et al., 2005; Turner, 2004; White et al., 1997).

Other evidence for the palliative treatment of inflamed gingiva among Pleistocene hunter-gatherers derives from interproximal grooves caused by dental probing or "toothpicking" (Lozano et al., 2013; Ungar et al., 2001). However, these features are also documented throughout the Holocene and are not always clearly associated with pathology (Brown and Molnar, 1990, Lukacs and Pastor, 1988;, Molnar, 2008; Molnar, 1971). By contrast, for Late Upper Paleolithic tooth extractions (i.e., avulsion or ablation) that were likely related to cultural modification of the dentition as an expression of social identities (Bocquentin, 2011; De Groote and Humphrey, 2016; Humphrey and Bocaege, 2008; Stojanowski et al., 2014; Willman et al., 2016). While not related to the treatment of pathology, ablation does offer evidence of invasive dental modifications in Late Upper Paleolithic contexts. Thus, toothpicking, caries manipulation, and ablation among Late Pleistocene hunter-gatherers experiencing high rates of dentognathic pathology (e.g., Capasso, 2011; Frayer, 1989; Humphrey et al., 2014; Lacy, 2014, 2015; Willman et al., 2016), suggest that the prerequisite stimuli (i.e., pathological affliction) and cultural practices for developing early dentistry practices may have much greater antiquity than currently documented.

Here we analyze two upper central incisors from a modern human recovered from the Late Upper Paleolithic site of Riparo Fredian (Molazzana, Lucca, Italy) (Boschian et al., 1995). Both I^1s exhibit antemortem modification to their pulp chambers in the form of striations and the presence of a composite material (bitumen and organic fibers) on the walls of the pulp cavities (Fig. 1). We provide a differential diagnosis for these features, and suggest that the modifications are intentional anthropogenic by-products of a pathology-induced therapeutic dental intervention.

[Figure 1 here].

Archaeological context

The Riparo Fredian is a mountainous area in northern Tuscany situated between the Alpi Apuane ridge to the west and the Apennines to the east. The site is located within the valley of the Turrite Secca River (in the territory of Molazzana, near Lucca), a tributary of the Serchio River (Fig. 2).

[Figure 2 here].

Thorough archaeological surveys carried out within the area brought to light several prehistoric settlements ascribed to the Late Upper Paleolithic (Late Epigravettian) and Mesolithic

(Sauveterrian and Castelnovian) (Biagi et al., 1981; Guidi, 1989; Tozzi, 1995). The results reveal that the area was completely abandoned during the Late Alpine Glacial, when the glacial fronts expanded downward to an elevation of about 700-800 m. The first groups re-entered the area during the Late Glacial Interstadial, and occupied sites at the bottom or on the lower sides of the valleys, whereas sites at higher elevation were not colonized until the Early Holocene. Riparo Fredian was found during these surveys, and systematic excavations were carried out from 1987 to 1990. It is situated on a river terrace about 2-3 m above the bottom of the valley, at about 360 m above sea level, and includes a habitation area of a few square meters.

The stratigraphic sequence (Fig. 2) is rather thin (1.60 m). The bottom of the sequence includes sandy river deposits (layers 8, 7 and 6), overlain by an archaeological sequence that includes Late Epigravettian (layer 5) and Mesolithic (layers 4 and 3) lithic industries. The sequence is terminated by thin lenses (layers 2 and 1) containing a few minute fragments of coarse pottery (Boschian et al., 1995). A cobble pavement of limited size was found at the top of layer 5 in the innermost area of the rock shelter (Fig. 3). This pavement included several large river cobbles that were irregularly distributed on a surface of about 2 m^2 and slightly protruded upwards into layer 4. Layer 4 also overlies layer 5 in the other areas of the shelter, where the two layers are in direct contact, and lack the cobble pavement. Most of the teeth found in the outer part of the cobble pavement were included in layer 4, whereas those found in the inside of the pavement were included mostly in layer 5. The following processes explaining the stratigraphic position of the human remains can be reconstructed by observing the architecture of the stratigraphic unit and the characteristics of the sediments.

[Figure 3 here].

An erosion process, subsequent to the formation of layer 5 but preceding the deposition of layer 4, eroded layer 5 on the outer side of the shelter and excavated a shallow trough. The cobbles of the outer part of the pavement slid into the trough and rotated towards the outside of the shelter and were found leaning slightly outwards. Sediments of layer 5, reworked by the erosion, accumulated into the trough together with the cobbles and formed the foundation for the outer part of layer 4. This process operated less intensely inside the rockshelter and reworked only the topmost part of layer 5, leaving the cobbles *in situ* and originating the inner part of layer 4, which is much thinner than the outer one. Consequently, layer 4 is thicker in the outer area of the shelter, whereas layer 5 is thicker in the inside area. As a result, layer 4 is largely composed of reworked parts of layer 5. Thus, it appears that the teeth were all originally embedded in layer 5, but those within the outer part of the cobble pavement were incorporated within layer 4 after reworking; conversely, those found in the inner part, where reworking was limited, remained *in situ* and hence were mostly associated with layer 5. Layer 5 was ^{14}C AMS dated on charcoal to 10,870±119 BP (AA10952, 13040 - 12600 cal BP for ±2σ calibrated range), and layer 4 to 9,458±91 BP (AA10951, 11106 - 10500 cal BP for ±2σ calibrated range).

The human remains from Riparo Fredian mostly consist of isolated teeth and these teeth have been attributed to six individuals (three subadults, three adults) based on dental anatomical features and levels of macroscopic wear (Boschian et al., 1995; Vierin, 2012). All of the teeth attributed to individual Fredian 5 (Fig. 4) were recovered from layer 5 next to an cobblestone artificially placed at its top (Boschian et al., 1995), which is attributed to the Final Epigravettian and dated by ^{14}C on charcoal between 13,040-12,600 cal BP (Boschian et al., 1995; D'Errico et al., 2011).

[Figure 4 here].

MATERIALS AND METHODS

The dental remains of Fredian 5.

Teeth 133 and 161 are right and left maxillary canines (C^1s), respectively. The occlusal cross-sections are asymmetrically oval, broad anteriorly, tapered distally, and the roots are long. Both C^1s have wear scores of 7 (Smith, 1984), but wear is slightly more advanced on the left C^1. Teeth 31 and 134, the subjects of the present study, are right and left maxillary central incisors (I^1s), respectively.

Siding is based primarily on the distolateral projection of the root apices. The right I^1 preserves a hairline rim of enamel on its anterior face (stage 7: Smith, 1984). The left I^1 is more circular in cross-section due to its greater degree of occlusal wear (stage 8: Smith, 1984). Both I^1 roots are mediolaterally and anteroposteriorly broad, a characteristic of maxillary central incisors that distinguishes them from the heavily worn C^1s and the maxillary second incisors (I^2s). Teeth 5 and 21 have been identified as right and left I^2s, respectively. The occlusal cross-sections are relatively round (compared to the canines and central incisors) and small in size. Siding is based primarily on wear associations between adjacent teeth. Each left tooth (134, 21, and 161) has a total length (root apex to occlusal surface) that is several millimetres less than that of their right-side antimeres.

Further evidence for tooth siding is provided through wear pattern associations. For instance, there is continuity in the wear planes and edge-rounding by side, which suggest that the behaviors resulting in wear differed between right and left sides of the mouth. The differential wear suggests that the left-side anterior teeth were used more extensively for masticatory and paramasticatory behaviors given that compensatory hypereruption would have kept the teeth in the same occlusal plane as the right-side anterior teeth despite progressive occlusal wear. However, the cause of differential wear is not immediately apparent. One possibility is that the anterior dental wear asymmetries may relate to the handedness of Fredian 5 during masticatory and non-masticatory behaviors. Another possibility is related to the timing of pulp exposure, infection, and subsequent antemortem modification of the pulp chambers. These explanations need not be mutually exclusive but are difficult to disentangle.

The subsequent analyses will focus on the pathological nature of the teeth as well as purported antermortem modifications indicative of probable dentistry.

MicroCT and digital reconstruction

High-resolution MicroCT images of the two upper central incisors were obtained with a Xalt MicroCT scanner (Panetta et al., 2012). All teeth were scanned at 50 kVp, 2 mm Al filtration, 960 projections over 360°, 0.9 mAs/projection for a total scan time of 50 minutes per sample. All the tomographic images were reconstructed using a modified Feldkamp algorithm (Feldkamp et al., 1984) with embedded compensation for mechanical misalignments and raw data pre-correction for beam-hardening and reduction of ring artifacts in the digital images. All images were reconstructed on a volume dataset of 600x600x1000 cubic voxels, each with a size of 18.4 μm. The image stacks were segmented using a semiautomatic threshold-based approach in Avizo 7 (Visualization Sciences Group Inc.) to distinguish between the dental tissues and the residue filling the pulp chamber as well as to reconstruct 3D digital models of the teeth.

Scanning electron microscope (SEM) and energy dispersion X-ray spectroscopy (EDS)

Back-scattered electron images and EDS spectra were collected on a low-vacuum ESEM FEI Quanta 200, equipped with an Oxford energy dispersive spectrometer. The analyses were conducted using an acceleration voltage up to 30 kV and EDS analyses performed at a working distance of 10 mm for 100 seconds. No sample preparation was required.

3D digital microscope

Multifocal images of anthropic cavities (up to 160X) were obtained using a Hirox KH-7700 Digital Microscope equipped with MX(G)-5040Z lens and an AD-5040LOWS adapter. Multifocal images of vegetal fibers as well as 3D images of microstriations (up to 7000X) were captured using a MX(G)-10C lens equipped with a OL-140II and OL-700II adapters and an AD-10S Directional Lighting Adapter. Multifocal and 3D images were created by overlapping a series of 120 photographs taken at different focus levels (Crezzini et al., 2014; Moretti et al., 2015). This procedure enables the observation of analyzed surfaces from different points of view, creation of cross-sections of the microstriations, and allows collection of linear, angular, and areal measurements (Boschin and Crezzini, 2012; Crezzini et al., 2014).

Fourier-Transformed Infrared Spectroscopy (FTIR)

FTIR spectroscopy was chosen because its sensitivity allows information to be gained from the small amount of material extracted from the teeth, which is otherwise insufficient for chromatographic analyses. Moreover, the advantages of FTIR (i.e., speed, economical and permits sample size) are added to the Attenuated Total Reflection (ATR) mode, which does not require sample preparation because the powdered sample is placed directly on the ATR prism. In this way, the impact preparation in KBr pellet and chemical alterations that may occur with chromatography are avoided (Hollund et al., 2013).

Once the incrustation of secondary dentine and matrix adhering at the bottom of the cavity was removed, FTIR-ATR was performed on the black film found inside the pulp cavities of both teeth. The samples were obtained with a scalpel scraping the inner surface subsequent to analysis of surface striae. Samples were also collected from the soil in which the teeth were embedded to control for possible contamination from exogenous materials.

FTIR analyses were performed in ATR mode with a Tensor 27 FTIR Spectrometer equipped with a diamond crystal. Spectra were recorded in the range of 4000-400 cm^{-1} at a spectral resolution of 4 cm^{-1} and 128 scans. Data acquisition was carried out using OPUS 7.2 software, the spectra were baseline corrected, the CO_2 was removed and a smooth performed.

Raman microscopy

A small amount of material containing the black patina encrusted on the internal surface of the teeth was investigated by Raman microscopy. The Raman spectra were collected with a Bruker Senterra Microscope interfaced with an Olympus microscope (20x-50x objective lens) fitted with a 785nm laser. The analyses were carried out with a 10mW laser power in the 50-2600 cm^{-1} spectral region and a resolution of 3 cm^{-1}.

Identification of the fibers

The samples were stained with the fluorochrome Calcofluor White M2R (Fluorescent Brightener 28, Sigma) that readily binds to cellulose and chitin. A working stock solution of 10 mg

ml^{-1} of Calcofluor white M2R was made in distilled water and then filtered through a 0.22 µm filter. The samples, mounted between slides and glass coverslips in distilled water, were treated with one drop of the Calcofluor solution. After removing the excess water, the presence of lignin was analyzed through acid Phloroglucinol staining (Phloroglucinol Sigma). The samples mounted between slides were treated with the stain (1% in ethanol) and then acidified with a drop of concentrated hydrochloric acid. The stained samples were observed under an inverted epifluorescence microscope Zeiss Axiovert 100, equipped with an UV filter (BP 365, FT 395, LP 397). The microscope was equipped with a Nikon color video camera Digital Sight DS-Fi2 with a DS-U3 control unit for image capture and Nis Elements-3 software was used for image analysis.

Radiocarbon dating

Fourier-Transform Infrared Spectroscopy

Both dentine and enamel from the Fredian 5 canine were analyzed with FTIR analysis to determine the state of preservation. A few dozen micrograms of dentine and enamel were separately powdered and homogenized in an agate mortar and pestle, mixed with a few milligrams of anhydrous KBr (Aldrich), and formed into a pellet. Infrared spectra were obtained at 4 cm^{-1} resolution Nicolet 380 FT-IR in transmission mode. The infrared splitting factors were calculated from the spectra following the method of Weiner and Bar-Yosef (Weiner and Bar-Yosef, 1990). The splitting factor for the enamel and dentine were 4.0 and 3.1, respectively. These values are in the range of well-preserved enamel and dentine (Asscher et al., 2011a,b). The FTIR spectrum of dentine mineral also showed absorption peaks at 1,651 cm^{-1} (amide I) and 1,556 cm^{-1} (amide II), indicating the presence of collagen clearly.

Dentine Collagen Extraction, Purification and Characterization

Some 193 mg of dentine was dissolved in 1N HCl to remove the mineral phase, centrifuged and rinsed three times in deionized water by centrifugation (6000 rpm for 2 min), and resuspension of the pellet. The pre-treatment procedure (Boaretto et al., 2009) for radiocarbon dating uses the acidalkali- acid (AAA) technique and filtration, after gelatinization, with Eezi filter and ultrafiltration (Yizhaq et al., 2005). Prior to the AMS (Accelerator Mass Spectrometry) target preparation the extracted collagen was analyzed with FTIR (Asscher et al., 2011a) The spectrum showed the three aminoacid peaks of amide I, II and hydroxyproline at 1650, 1550 and 1450 cm^{-1}, respectively. No other minerals were detected.

Target Preparation and AMS Analysis

The extracted collagen sample RTD-8546 was combusted to CO_2 in vacuum sealed quartz tubes containing approximately 200 mg of copper oxide (Merck) and heated to 900°C for 200 minutes. The CO_2 was divided into 3 aliquots and then each was reduced to graphite using cobalt (Fluka) (approximately 1mg) as a catalyst and hydrogen, and heated to 700°C for 20 hours. The graphite produced was analyzed for ^{14}C content at the D-REAMS Radiocarbon Laboratory at the Weizmann Institute. Calibrated ranges in calendar years have been obtained from calibration tables (Reimer et al. 2013) by means of OxCal v4.2.4 (Bronk Ramsey and Lee, 2013).

RESULTS

Both upper central incisors are heavily worn with occlusal exposure of each pulp chamber (RI^1: mesio-distal =2.82 mm; labio-lingual=3.08 mm; LI^1: mesio-distal=2.77 mm; labio-lingual=2.84 mm). The pulp chambers show a rounded perforation (hereafter called “cavity”) that appear to be circumferentially (albeit unevenly) enlarged (Fig. 1a, d) and extend into the root for 4.82 mm (RI^1) and 4.25 mm (LI^1), with a sudden transition with the preserved portion of the pulp canal, which is partially filled with organic residue (Fig. 1c, f). Scanning Electron Microscopy (SEM) analysis showed microwear in the form of small scratches on the polished incisal surface and occlusal margins of the cavities (Fig. 5).

[Figure 5 here].

Additional SEM analysis revealed striations in the internal cavity surface (Fig. 6), which differ from the typical dental microwear pattern, along with two dentine chips on the lingual (RI^1) and labial (LI^1) margins, respectively (Fig. 1a, d). The margins of the chipped dentine exhibit smooth and rounded edges, similar to antemortem enamel chipping (Bonfiglioli et al., 2004; Scott and Winn, 2011), which indicates some degree of *in vivo* occlusal wear and tool-use following exposure of the pulp cavity and the chipping of the dentine. Together, the scratches and rounding of the dental chips on the margins of the cavities suggest that Fredian 5 survived initial pulp exposure and continued to use their anterior teeth for daily activities prior to death.

[Figure 6 here].

The striations on the internal surfaces of the pulp cavities are distinguished from the scratches on the occlusal surface by a difference in orientation and by a distinct morphological appearance. The shape and cross-section of the striations are diagnostic of the instrument used to produce them and the activities involved. Some are “V” shaped in transverse section and have a combination of attributes similar to the recognition criteria of slicing cut marks (Fig. 6d) (morphological categories 2, 4 and 5 [Boschin and Crezzini, 2012]) produced by stone tools, while others are shallower with more rounded cross-sections (Fig. 6b, f). The latter resemble those produced during experimental tests in dentine with bone tool (Oxilia et al., 2015).

The residue filling the pulp canals was removed and analyzed by SEM and stereomicroscopy. SEM analysis shows the presence of dentinal tubules, suggesting the residue has extensive dentine adhering to it postmortem (Supporting Information Figure S1). Moreover, a number of microscopic materials with a fibrous-like morphology were found; however, only a few could be isolated due to their small dimensions and fragmented state. The fibers were observed using an optical microscope and examined by means of histochemical methods. Two main morphological classes were documented. The Type 1 fiber had a length of 51.56 µm and an irregular width with a mean diameter of 24.4 µm (Fig. 7a). It was flexible with some distinct folds and reacted with the staining specific for cellulose and chitin (Fig. 7b), but not with the one specific for lignin. Due to the size and morphology, this fiber type was more consistent with a plant fiber classification rather than fungi. The Type 2 fiber had a light brown pigmentation, a round morphology with a diameter of approximately 60 µm, and was also flexible but seemingly hollow (Fig. 7c). This fiber did not react with either cellulose (Fig. 7d) or lignin (Fig. 7e) stains. The size, morphology and histochemical results obtained from this fiber suggest it should be classified as hair.

[Figure 7 here].

Fourier transform infrared spectroscopy (FTIR) analysis was carried out on the black patina adhering to the inner walls of the cavities and on the soil from the deposit from which the teeth were retrieved. First, it was possible to discard external contamination as the soil analyses showed a composition of calcite, silicates and quartz (Supporting Information Figure S2). The FTIR spectra obtained on the black patina are similar in both samples (Fig. 8). The peaks at 1022, 600 and 562 cm^{-1} (stretching and bending modes of PO_4) are related to hydroxyapatite, due to the contamination of dentine adhering to the black patina. Furthermore, the sharp and strong peaks at 2922 (CH_3 bending bond) and 2850 cm^{-1} (CH_2 bending bond) and the weak peak at 2956 cm^{-1} show the presence of organic matter with strong absorption of aliphatic CH. The lack of a defined peak in the 1750-1650 cm^{-1} region suggests the organic material does not have a carbonyl group, thereby excluding the presence of oil, wax, gums, natural resin or proteinaceous material, such as egg or animal glue (Daher et al., 2010; Derrick et al., 1999). According to previous studies (Cârciumaru et al., 2012; Hassan et al., 2013; Lamontagne et al., 2001), the two characteristic peaks at 1472 and 1382 cm^{-1} could indicate the presence of CH_2 and CH_3 bending bonds, respectively. The closest spectral match is with a reference spectrum gained from the IRUG online database (Harvard University Database, 2016) and is ascribable to bitumen.

[Figure 8 here].

A Raman spectrum was additionally acquired on the internal surface of the pulp cavities to distinguish the characteristic peaks of hydroxyapatite at 962 cm^{-1} (Supporting Information Figure S3). The spectrum of interest on the black patina, instead, shows broad peaks around 1305 and 1595 cm^{-1}, which can be associated with amorphous carbon, probably attributable to bitumen.

Bitumen is an organic material with a very complex chemistry (Vandenabeele et al., 2007) because it is a mixture mostly of hydrocarbons with a small number of heterocyclic species and functional groups containing sulphur, nitrogen and oxygen. Accordingly, the energy dispersion X-ray spectroscopy (EDS) spectra were acquired on a small grain of material containing the black patina encrusted on the tooth's inner surface. An increasing degree of carbon (C) and the presence of sulphur (S) and nitrogen (N) were found in addition to the elements related to the chemical composition (Ca, P, O) of the teeth (Supporting Information Figure S4). This result can therefore be explained by the presence of sulphur, nitrogen and oxygen in the bitumen composition as heterocyclic atoms (McNally, 2011).

A direct radiocarbon date for Fredian 5 was obtained from the dentine of the right canine (RTD 8546) (Supporting Information Figure S5; Supporting Information Table S1). The new radiocarbon date, 11,000±40 ^{14}C year BP is well in the range of the Epigravettian period with a 95.4% probability calibrated range of 13,000-12,740 cal BP.

DISCUSSION

Fredian 5 exhibits occlusal pulp exposure of both I^1s, but this affliction is not an unusual occurrence among Late Upper Paleolithic hunter-gatherers (e.g., Capasso, 2001; Da-Gloria and Larsen, 2014; Lieverse et al., 2007; Lukacs, 1988; Porr and Alt, 2006) that warrants further explanation. However, the internal surface modifications to the pulp cavities, in addition to the presence of bitumen and organic fibers, is an unusual occurrence among Late Upper Paleolithic hunter-gatherers that begs further explanation. Caries manipulation was previously recorded in the penecontemporaneous (Epipaleolithic) individual Villabruna 1 individual (Oxilia et al., 2015), suggesting that the presence of the above features could be the result of similar pathology manipulation in Fredian 5. Thus, we offer a differential diagnosis for the suite of characteristics associated with the pulp cavity modifications documented for Fredian 5. We have identified four possible diagnoses: 1) Postmortem/Taphonomic Modifications; 2) Ingestive Behaviors and Teeth-as-Tools; 3) Cultural Modification for Social Expression; and 4) Therapeutic Dentistry. We explore each diagnosis in detail below and discuss potential overlap between them.

1. Postmortem/Taphonomic Modifications

The exposed pulp chambers are undoubtedly antemortem, but the extent to which the markings on the internal surface of the pulp cavities are of antemortem versus postmortem origin must be explored further. Dental drilling tends to produce parallel striations or microgrooves around the circumference of the drilled cavity (e.g., Bennike and Alexandersen, 2003; Coppa et al., 2006; Ortiz et al., 2016; Schwartz et al., 1995; Seidel et al., 2005; Turner, 2004; White et al., 1997). The case of Fredian 5 shows less-intensive markings than documented in chronologically more recent examples of dental drilling and the striations are parallel to the horizontal axis of the tooth. These markings would be consistent with the twisting of a hard implement (e.g., bone or lithic) placed inside the pulp cavities, and are similar to the striations created by the scraping and levering actions during caries manipulation in Villabruna 1 (Oxilia et al., 2015). The same forms of striations are not found on the occlusal external root surfaces of the Fredian 5 anterior teeth. If the markings on the internal surface are the product of postmortem damage caused by cleaning, we would expect to see similar marking on the occlusal surfaces, but there are no such markings. Therefore, we find it difficult to explain how a postmortem process could preferentially leave marking on internal surfaces while leaving the external surfaces unmarked.

Moreover, bitumen is known to have been used as hafting compound by Pleistocene foragers from Middle and Upper Paleolithic contexts (Boëda et al. 1996, 2008; Cârciumaru et al., 2012). Bitumen, along with other hafting materials (e.g., pitch and resin), have been documented in museum collections derived from decades old excavations (Cârciumaru et al., 2012; Dinnis et al., 2009), which attests to the possibility of long-term preservation of such residues following excavation, repeated handling, and curation. It is difficult to explain how a postmortem processes that would cause an organic substance such as bitumen to be preferentially deposited (and preserved) only inside the two pulp cavities, but be absent on the external surfaces of the teeth and surrounding archaeological matrix from the site (see Results). Consequently, we view a scenario in which postmortem, taphonomic processes caused the modifications to the Fredian 5 pulp cavities as unlikely.

2. Ingestive Behaviors and Teeth-as-Tools

The exposure of pulp chambers through attrition in hunter-gatherers is not uncommon, for it is often found among foragers with extensive anterior tooth wear caused by a combination of ingestive food processing behaviors and non-masticatory uses of the "teeth-as-tools". In the case of Fredian 5 it is evident from the presence of fine occlusal striations and rounding of the dentin chips around the exposed pulp cavities that the I1s continued to be used after pulp exposure for ingestive and/or non-masticatory behaviors.

Given the presence of occlusal wear following antemortem pulp exposure there is a possibility that the striations inside the pulp cavities could have been caused by continued anterior tooth-use. For instance, some hunter-gatherers retouch the working-edge of lithic implements with their anterior teeth (Gould, 1968), a process that could introduce microflakes into exposed pulp cavities. Grit, bone fragments, and other abrasive materials from food or various materials worked between the anterior teeth (e.g., wood, hide, plant and animal fibers) could also have entered the exposed pulp cavities of Fredian 5 unintentionally. With this scenario, the foreign materials or debris entering the pulp cavities would had to have moved along a horizontal plane to produce the striations documented in the Fredian pulp cavities, but such movements are unlikely to be produced by the vertical motions and compressive forces of the teeth and jaws during ingestive and/or non-masticatory behaviors. Rather, such striations are more likely to have been induced by movements that involved twisting and scraping an implement along a horizontal axis within the pulp cavity. A lack of dietary microwear within the cavities also suggests that mastication was unlikely to have contributed greatly to the expansion of the cavities. The most parsimonious explanation is that Fredian 5, perhaps with the assistance of another individual, intentionally manipulated an object that produced horizontal striations on the internal walls of the cavity.

Support for this interpretation comes from Villabruna 1, which also lacked dietary microwear deep within the manipulated caries but does present distinctive, tool-induced striations within the margins of the caries (Oxilia et al., 2015). Furthermore, experiments show that striations similar to those in the present (in shape, cross-section, and orientation [horizontal]) are produced through levering and twisting actions (Oxilia, et al., 2015 SOM).

If Fredian 5 used their anterior dentition to manipulate implements covered in bitumen (e.g., items waterproofed with bitumen or hafted objects), then it is also likely that the occlusal surfaces of the I^1s would be more extensively impregnated with bitumen. Instead, only the edges of the exposed pulp cavities, the internal surfaces, and the deep recesses within the pulp canal are infilled with bitumen. Furthermore, there are no traces of bitumen on the occlusal surfaces of the other four anterior teeth of Fredian 5 despite their similar states of wear. If the bitumen in the pulp cavities entered unintentionally, we expect that traces of bitumen on the occlusal surfaces to be present on all six anterior teeth, not just within the pulp cavities of the I^1s. The majority of the residue is found deep in the pulp canal rather than distributed throughout the entire pulp chamber/cavity, and it is notable that no bitumen is found embedded recesses of the antemortem enamel and dentin chips on either tooth. We expect that the accumulation of residue through unintentional causes would not limit the majority of bitumen accumulation to the pulp canals, and that the occlusal recesses caused by chipping would be more likely to retain remnants of bitumen even after continued dietary and non-masticatory tooth-use. Given neither circumstance is recorded in Fredian 5, we find the presence, location, and preservation of bitumen in the pulp canals difficult to explain without invoking explicit anthropogenic intentions.

The orientation of the striations inside the pulp cavities suggests intentional movements of an extraneous implement, while the presence of bitumen inside the pulp cavities, but no other surfaces, also suggest intentional placement of the bitumen. However, it is much more difficult to rule out an unintentional origin of the vegetal and hair fibers in the pulp cavities. These materials could have been unintentionally adherent to the bitumen when it was placed in the cavities, regardless of whether bitumen was entered through dietary or non-dietary behaviors, or intentionally placed inside the cavities. Given the degraded characteristics of the fibers, and the low number recovered, we cannot rule out their presence as an unintentional result of dietary and/or non-masticatory behavior. Therefore, we suggest intentional behaviors produced the internal pulp cavity striations and presence of bitumen, but we cannot determine intentionality for the presence of organic fibers in the pulp cavities definitively.

3. Cultural Modification for Social Expression

Regional traditions of intentional dental modification for purposes of cultural expression of social identities are well documented in the Late Upper Paleolithic and are best represented by the practice of dental ablation throughout North Africa, Southwest and Southeast Asia, and Australia during the Late Pleistocene (see review in Willman et al., 2016). However, ablation generally leaves large gaps in the dental arcade due to the tooth removal that disrupt for patterns of occlusion and dental wear (Humphrey and Bocaege, 2008). Occlusal wear is relatively even and extensive across all six of the maxillary anterior teeth of Fredian 5, which suggests that the individual's mandibular isomeres were present (i.e., not ablated) and in occlusion. Using the same logic, we can rule out ablation through "tooth-knocking" (i.e., breaking the crown off at the cervix: Pietrusewsky and Douglas, 1993), and add that there are no signs of root resorption (Fig. 4) typical of traumatic fracture (Lukacs, 2007).

The filing of anterior dental crowns into specific shapes to express aspects of social identity is well-documented from prehistory into the ethnographic present (Alt and Pichler, 1998; Fastlicht, 1976; Milner and Larsen, 1991; Stojanowski et al., 2016; Tiesler, 2011), and provides an alternative for the dental modification found in Fredian 5. However, to date there is only one case of abrasive wear from a Late Upper Paleolithic context that resembles filing (Bocquentin et al., 2013). The case concerns flattened and polished labial enamel on the upper central incisors of an Early Natufian individual from Jordan, but the wear cannot be definitively attributed to the use of teeth-as-tools or an intentional marker of social identity (Bocquentin et al., 2013). Filing generally involves shaping of the crown without removal of the entire crown (e.g., Alt and Pichler, 1998; Fastlicht, 1976; Milner and Larsen, 1991; Stojanowski et al., 2016; Tiesler, 2011), which is inconsistent with the complete loss of crowns in Fredian 5.

A last possibility for cultural modification of social expression would be that the pulp chambers were modified, drilled, or otherwise expanded for the inclusion of a foreign object (e.g., inlays), although this is unlikely for a number of reasons. First, inlays are generally associated with drilling into the labial surfaces of teeth to prepare for the placement of decorative inlays as this would be readily visible (Alt and Pichler, 1998; Fastlicht, 1976; Milner and Larsen, 1991; Tiesler, 2011), and there are no documented cases of decorative inlays being placed in modified pulp chambers/cavities in the archaeological or ethnohistorical literature to our knowledge. Second, while drilling to prepare inlays shares technological attributes with the drilling procedures used for therapeutic purposes (Bennike and Alexandersen, 2003; Coppa et al., 2006; Ortiz et al., 2016; Schwartz et al., 1995; Seidel et al., 2005; Turner, 2004; White et al., 1997), there is no evidence for this form of extensive drilling prior to the Neolithic (Coppa et al., 2006). Moreover, an exposed and modified pulp cavity would have been sensitive to non-therapeutic inclusions such as inlays or other decorative objects, when subjected to any compressive forces during masticatory and/or non-masticatory behaviors. Lastly, the presence of occlusal wear and rounded edges of dentine chips provides evidence for the continued use of the well-worn roots as a functional occlusal surfaces before death. If foreign objects were placed in the pulp cavities for cultural/aesthetic purposes one would not expect to see microwear related to normal tooth-use on the occlusal surfaces. We therefore suggest that an antemortem/cultural expression scenario is unlikely to explain the modifications to the I^1 teeth of Fredian 5.

4. Therapeutic Dentistry

A final possibility for the presence of a suite of antemortem modifications of the I^1 pulp cavities of Fredian 5 dentition may be through a therapeutic dental intervention. Pulp exposure is commonly associated with severe anterior dental attrition among foragers (Da-Gloria and Larsen, 2014; Lieverse et al., 2007; Lukacs, 1988; Porr and Alt, 2006), and high rates of oral pathology have recently become well documented among Terminal Pleistocene foragers (e.g., Capasso, 2001; Frayer, 1989; Humphrey et al., 2014; Lacy, 2014, 2015; Willman et al., 2016). These rates of pathology suggest a precedent for exploring pathology-induced dental interventions was present among Late Upper Paleolithic foraging groups. Similarly, the recent documentation of dental manipulation associated with pathology (caries) in the Late Upper Paleolithic Villabruna 1 fossil (Oxilia et al., 2015) suggests that other early cases of dental intervention may yet be waiting to be documented.

Villabruna 1 exhibits striations consistent with scraping, levering, and probing an occlusal surface caries on a mandibular third molar – remnants of behaviors that partially removed the caries (Oxilia et al., 2015). Fredian 5 exhibits a degree of intentional modification that is similar to that of Villabruna I. The Fredian 5 pulp cavities exhibit horizontal striations produced by scraping and twisting actions of a sharp, hand-held implement that resulted in circumferential enlargement of the cavities (in comparison to recent dental drilling interventions). The similarities between the striations in the two specimens suggests intentional, manually-implemented, behaviors rather than unintentional byproducts of tooth-using behaviors, aesthetic modifications, or taphonomy.

Crediting a motive to the intentional dental modifications in Fredian 5 is made more difficult by the considerable differences in the form of the modifications compared to other documented cases from the Holocene – namely those that involved drilling for probable therapeutic purposes (Bennike and Alexandersen, 2003; Coppa et al., 2006; Ortiz et al., 2016; Schwartz et al., 1995; Seidel et al., 2005; Turner, 2004; White et al., 1997). The use of levering and scraping in Villabruna 1, rather than drilling, can be explained by the distal position of the carious lesion in the oral cavity (of the right M_3). This is noteworthy because there are no documented cases of third molar dental drilling in more recent contexts (Bennike and Alexandersen, 2003; Coppa et al., 2006; Ortiz et al., 2016; Schwartz et al., 1995; Seidel et al., 2005; Turner, 2004; White et al., 1997).

In contrast to Villabruna 1, access to the I^1s of Fredian 5 would not preclude a more invasive drilling intervention like those found in many Holocene context. Nevertheless, the subtle horizontal striations and circumferential enlargement of the cavities do show clear evidence intentional manipulation. However, additional concentric striations may be obscured by remnants of bitumen. Indeed, no bitumen was associated with the Villabruna 1 caries and the striations associated with caries manipulated are much clearer (Oxilia et al. 2015).

Numerous other explanations could account for the subtle nature of the horizontal striations in the Fredian 5 pulp cavities (e.g., some striations were erased through later abrasive wear – from removing and reapplying an organic filling, or from food and other debris entering the cavity following the initial use of bitumen). Although it is also probable that the intervention was simple less-invasive than those documented from more recent contexts.

The subtle markings from Fredian 5 (and to some extent, Villabruna 1) are infrequently documented compared to the obvious drill-induced modifications from the Holocene, but this infrequency may be biased due to the ease of identification in the latter cases. Indeed, the subtle modifications to the pulp chambers of Fredian 5 and caries manipulation of Villabruna 1 were difficult to observe macroscopically, and required extensive microscopic, microCT, and residue analyses to completely characterize. Consequently, the subtle manipulation of pathologies in the two cases from the Italian Epigravettian suggest that Holocene case studies of purposeful drilling should not be used as baseline characteristics for all pathology-induced dental interventions. It is probable that additional cases have gone undocumented given no reference for identifying the subtle modifications of Fredian 5 and Villabruna 1 existed until recently.

The presence of bitumen in the pulp cavities of Fredian 5 is an additional unique finding that is most likely explained by a therapeutic diagnosis. The lack of bitumen on any surface other than the inside of the pulp cavities is suggestive of intentional placement. Uses of bitumen are not unknown in the Paleolithic (Boëda et al. 1996, 2008; Cârciumaru et al., 2012), but have not been documented on dental surfaces prior to this study. However, residue and microfossil studies of dental surfaces are relatively recent innovations in paleoanthropology and unique discoveries have been made in most studies to date (Hardy et al., 2012, 2016; Henry et al., 2011; Radini et al., 2016). The presence of bitumen (and horizontal striations) inside the pulp cavity but not on other surfaces of the teeth suggests intentionality in their placement in the cavities. Therefore, the bitumen and pathological exposure of the pulp chambers through attrition may likely have been therapeutic.

While it is speculative in the present study, the use of bitumen could have been used as an antiseptic or to provide an anti-microbial barrier between the body and the environment (Bourée et al., 2011; Connan, 1999). A similar suggestion has been made for a Neolithic beeswax filling (Bernardini et al., 2013). Furthermore, the presence of hair and plant fibers could indicate the use of a composite filling material, but there is no way to be certain that the hair and vegetal fibers were purposefully placed in the cavities like the bitumen likely was. However, the probable use of medicinal plants is not without precedence in the Pleistocene (Hardy et al., 2012) and early Holocene (Aveling and Heron, 1999). There is also ample ethnographic documentation of plants used for the treatment of toothaches, caries, pulpitis, and other ailments (Buckley et al., 2014; Elvin-Lewis, 1982, 1986; Moerman, 1998; Willey and Hofmann, 1994).

CONCLUSIONS

Given the evidence for probable dentistry in Fredian 5 it is now possible to suggest that the caries manipulation found in Villabruna (Oxilia et al., 2015) may be part of a broader trend, or tradition, of pathology-induced dental interventions among Late Upper Paleolithic Italian foragers. Both Fredian 5 and Villabruna 1 represent cases where implements were used to manipulate dental pathologies. The Late Pleistocene is a period of increasingly diverse and broad spectrum socioeconomic activities. The concomitant increase in dentognathic pathology likely called for novel strategies to cope with changing morbidity profiles. Thus, this discovery marks a much earlier instance of pathology-induced therapeutic dental interventions than previously known.

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