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Beyond food: The multiple pathways for inclusion of materials into ancient dental calculus

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Abstract
Dental calculus (mineralized dental plaque) was first recognised as a potentially useful archaeological deposit in the 1970s, though interest in human dental calculus as a resource material has increased sharply in the past few years. The majority of recent research has focused on the retrieval of plant microfossils embedded in its matrix and interpretation of these finds as largely the result of deliberate consumption of plant-derived food. However, while most of the material described in published works does represent food, dental calculus is in fact a "depositional environment" as material can enter the mouth from a range of sources. In this respect, it therefore represents an archaeological deposit that can also contain extensive non-dietary debris. This can comprise a wide variety of cultural and environmental material which reaches the mouth and can become embedded in dental calculus through alternative pathways. Here, we explore the human behaviors and activities besides eating that can generate a flux of particles into the human mouth, the broad range of additional cultural and environmental information that can be obtained through the analysis and contextualisation of this material, and the implications of the additional pathways by which material can become embedded in dental calculus.

KEYWORDS
dental calculus, diet, environment, microfossils

1 | INTRODUCTION

Dental calculus results from the calcification of plaque and can be retrieved from skeletal material dating to most archaeological periods (Figure 1). Indicative of its preservation potential is the fact that it has been found on the teeth of a Miocene *Sivapithecus* dating to between 12 and 8 million years ago (Hershkovitz et al., 1997) as well as on late Pliocene hominins (Blumenschine et al., 2003). It has become increasingly clear that dental calculus is a store of a wide range of *in situ* biographical information, as it acts as a trap for microscopic fragments of debris. These debris may include human cells, mineralized bacterial structures and plant microfossils as well as chemical and biomolecular compounds that have passed through the mouth during life (Buckley, Usai, Jakob, Radini, & Hardy, 2014; Blondiaux & Charlier, 2008; Charlier et al., 2010; Dobney, 1994; Dobney & Brothwell, 1987; Hardy et al., 2012, 2015; Vandermeersch et al., 1994; Warinner, Speller, Collins, & Lewis, 2015). This has led, unsurprisingly, to a growing interest in dental calculus as a source of direct evidence for ancient biographical and dietary information.

As dental calculus forms in the human mouth, the assumption so far has primarily been that much of this material represents food consumed, and therefore offers direct information on items that were intentionally eaten (e.g., Radini, Nikita, & Shillito, 2016). However, it is now becoming increasingly clear that the material found embedded in dental calculus can come from a range of different sources. The calculus matrix may be enriched with plant and other remains derived from accidental inhalation or ingestion of non-dietary debris, oral hygiene activities and use of the mouth as a third hand (e.g., Blatt, Redmond, Cassman, & Sciulli, 2011; Blondiaux & Charlier, 2008; Buckley et al., 2014; Charlier et al., 2010; Hardy, 2016; Hardy et al., 2016; Radini, Buckley, et al., 2016). The need to be aware of the complex interaction between people and plant resources when reconstructing plant use in past human activity has been discussed previously (Beck & Torrence, 2006); however, though non-dietary debris in particular can be

FIGURE 1 Human dental calculus on a male individual from the Medieval Parish of St. Michael's, Leicester 1250–1400 AD

challenging to identify, there is a need to consider all possible pathways for the inclusion of materials into dental calculus, in addition to deliberate ingestion.

In the light of this, we examine the use of microfossils extracted from dental calculus in ancient dietary reconstruction, and we explore the many non-dietary pathways whereby micro-debris can become embedded. We highlight the need to contextualise all results within their natural and cultural environments and we conclude by suggesting that the real value of dental calculus, as a store for a wide range of biographical and environmental data, has yet to be fully exploited.

2 | FORMATION PROCESS, STRUCTURE AND COMPOSITION OF DENTAL CALCULUS

2.1 | Formation process

Dental calculus forms on teeth through a complex interaction between saliva and bacteria on the dental surface. Saliva is made up of water (99.5%), electrolytes, mucus, antibacterial compounds, enzymes and bacterial cells (Roberts, 1979). Saliva lubricates the mouth and moistens food to create a bolus, which is then swallowed. It contains the enzyme α-amylase, which commences the breakdown of starch into simple sugars and also immunoglobulins, which control the microorganisms in the mouth and can restrict the build-up of plaque (de Almeida, Grégio, Machado, de Lima, & Azevedo, 2008). Saliva is primarily formed by three pairs of glands, the parotid glands, the submandibular glands and the sublingual glands, and empties into the mouth through their respective ducts. The parotid gland ducts are located inside the cheeks near the upper molars, while the submandibular and sublingual gland ducts are located under the tongue (Edgar, O'Mullane, & Dawes, 1996).

The mouth contains many species of Gram-positive and Gram-negative bacteria (Hillson, 2005), including spherical cocci (e.g., streptococci and staphylococci), and rod-shaped bacilli (e.g., lactobacilli and

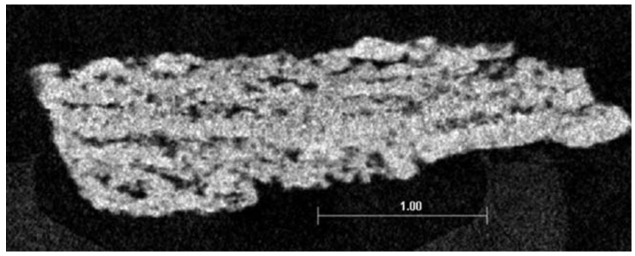

FIGURE 2 Micro CT scan of dental fragment of calculus (from Hardy et al., 2013)

corynebacteria). When food is chewed, plaque is formed by the adsorption of proteins and bacteria, predominantly the facultative anaerobe *Streptococcus mutans* (Marcotte & Lavoie, 1998). These metabolise salivary sugars to form a film that adheres to the surface of the tooth. The bacteria are rapidly replaced by calcium phosphate salts and if the plaque is not cleaned off, microorganisms nearest the tooth surface ferment sugars in the saliva and produce acids that demineralise the tooth, ultimately producing caries (Hillson, 2005). The plaque hardens rapidly, beginning with the cell walls of the bacteria (Hillson, 2005) and can be fully calcified within 2 weeks (Lieverse, 1999). The rough surface of dental calculus serves to attract other bacteria which adhere to those already attached, while the gaps between the cells are filled by other components of the saliva to produce a layered structure (Figure 2) (Dobney & Brothwell, 1987; Hardy, van de Locht, Wilson, & Tugay, 2013). The build-up of dental calculus can be greatest near the salivary ducts; this results in deposits that are more prominent on the lingual surfaces of incisors and canines and the buccal surfaces of maxillary molars (Hillson, 2005). However, calculus is not restricted to these locations and in extreme cases it can almost cover all teeth.

When food is introduced into the mouth, the mechanical process of chewing can lead to material becoming entrapped in the gingival crevice of the teeth (Figure 3). The bacteria in the gingival crevice are predominantly proteolytic (utilising protein as substrate rather than

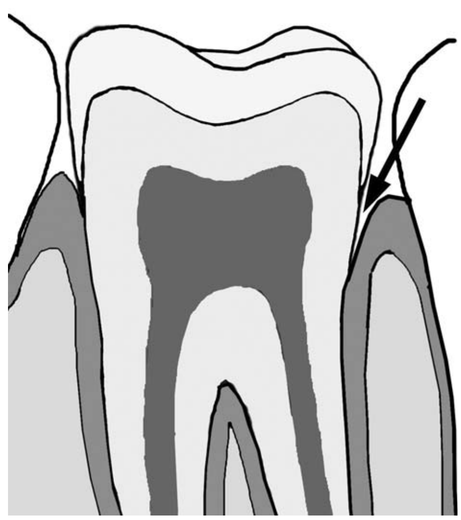

FIGURE 3 Location of subgingival crevice on teeth

sugars, contrary to those in the mouth, which are saccharolytic); this favors the preservation of starch granules once they have reached the gingival crevice (Marcotte & Lavoie, 1998). The resulting metabolic by-products of proteolytic metabolism, such as ammonia, result in localized raised pH, which in turn favors plaque mineralization by stimulating precipitation of calcium phosphate. The antimicrobial constituents of the saliva (e.g., immunoglobulins and degradative enzymes) break down the microorganisms that have survived in the mouth (Marcotte & Lavoie, 1998). However, some material escapes breakdown and can become covered with plaque within hours. It is then protected from the effects of the α-amylase and becomes embedded in the calculus matrix (Marcotte & Lavoie, 1998; Scannapieco, Torres, & Levine, 1993).

Dental calculus has a multicausal etiology. Until recently, it was widely assumed that calculus deposits indicated diets rich in protein since such diets increase oral alkalinity, which in turn facilitates calculus formation (Hillson, 1979; Lillie & Richards, 2000; Meiklejohn & Zvelebil, 1991; but see Lieverse, 1999 for a review of calculus formation processes). It is now clear that the dietary information provided by the extent of calculus deposits is not as straightforward as originally assumed. Specifically, high calculus deposition combined with low incidence of caries has been considered to suggest a high protein intake (Keenleyside, 2008; Lillie, 1996), whereas occurrence of both high calculus and caries is understood to predominate in diets high in carbohydrates (Humphrey et al., 2014; White, 1994). In any case, non-dietary factors such as the rate of salivary flow, mineral and silicon content consumed in food and water, phosphate and calcium levels in the blood and genetic factors also influence the occurrence of calculus deposits, possibly more so than diet (Lieverse, Link, Bazaliiskiy, Goriunova, & Weber, 2007). Finally, mechanical factors, such as chewing, have a contradictory effect; the act of chewing may promote calculus formation by increasing salivary flow rate (Dawes, 1970), whereas chewing abrasive materials may mechanically remove calculus deposits (Gaar, Rølla, & van der Ouderaa, 1989).

Besides the multifactorial nature of calculus formation processes and the challenge this can cause to the interpretation of the embedded micro-debris, additional issues arise from the fact that the morphological variation of teeth, expressed in the co-existence of smooth surfaces, fissures and pits, creates different surface morphologies that facilitate or hinder calculus formation (Hillson, 2005). Furthermore, within each plaque deposit there may be marked variation in pH, nutrient availability, and temperature, which can affect the formation and structure of the calculus (Hillson, 2005).

2.2 | Relevant aspects for archeology

There are three main aspects of the dental calculus formation process most relevant to archeology. First, and most importantly, the process of calculus formation ceases at death, therefore it has high archaeological integrity as postmortem inclusions of micro-debris from soil are very unlikely (Middleton & Rovner, 1994).

The second aspect, and the one mostly considered to date, is that dental calculus can entrap debris, microorganisms, molecules of various types that enter the mouth during the life of an individual, providing a novel archaeological record (e.g., Buckley et al., 2014; Radini, Buckley, et al., 2016; Radini, Nikita, et al., 2016; Warinner, Rodrigues, et al., 2014; Warinner, Hendy, et al., 2014).

Thirdly, the rate of calculus formation is variable and associated with differences in diet among individuals, salivary flow rate, health of the individual, local pH and genetic factors (Marcotte & Lavoie, 1998). There continues to be no clear understanding of when and how fast dental calculus builds up on the teeth of different individuals, or how much of an individual's life may be represented in this matrix, which means that comparative and quantitative approaches are currently not possible (e.g., Leonard, Vashro, O'Connell, & Henry, 2015; Power, Salazar-García, Straus, Morales, & Henry, 2015).

Despite this limitation, the direct connection of dental calculus to individual ingestion as well as its ubiquity in past populations means that it has extraordinary potential for investigating details of past lives, both at individual and population levels.

3 | DENTAL CALCULUS AND ANCIENT DIET

In archeology, the study of dental calculus has been approached macroscopically (quantity and location of calculus deposits on teeth), microscopically (debris entrapped in it), and more recently, biomolecularly. Early work on dental calculus focused on macroscopic quantification and location of calculus found (Dobney & Brothwell, 1987) and this approach is still sometimes used (e.g., Humphrey et al., 2014; Jankauskas & Palabeckaite, 2006; Keenleyside, 2008; Lillie, 1996). Different recording protocols have been proposed for this purpose, ranging from simple presence/absence (e.g., Belcastro et al., 2007) to more detailed ordinal schemes (Brothwell, 1981; Dobney & Brothwell, 1987) or even continuous schemes that measure the maximum extent of the deposit (Hillson, 2000). Such studies generally treat the amount of dental calculus per individual, combined with observations on other dental diseases such as caries, as dietary indicators as well as markers of oral health and oral hygiene. A major limitation in this approach is that dental calculus deposits, particularly the supragingival ones, can detach from the teeth both during life and postdepositionally (Buikstra & Ubelaker, 1994), which can bias the results. Detachment is less common for subgingival deposits as these are protected to some extent by the gum (Figure 3), unlike supragingival deposits, which accumulate on the exposed surfaces of the teeth.

The potential of dental calculus to be a trap for a variety of microscopic dietary and environmental micro-debris was first recognized in 1975 when phytoliths, opaline silica deposits that form in certain types of plants, were extracted from samples of ungulate dental calculus (Armitage, 1975). Dobney and Brothwell (1987, 1988) were the first to explore the potential for obtaining information on archaeological samples of dental calculus, using scanning electron microscopy (SEM) to identify pollen grains, phytoliths, charcoal, microscopic fragments of cereal chaff and animal hairs from a range of different historical and archaeological material. Following this, using SEM, fossilised bacteria were detected in the calculus matrix of Neanderthal (Pap, Tillier, Arensburg, Weiner, & Chech, 1995; Vandermeersch et al., 1994), Natufian (Arensburg, 1996) and multi-period Chilean samples (Linossier, Gajardo, & Olavarria, 1996). A more recent SEM analysis identified microfossils related to diet, including palm phytoliths while diatoms were used to examine water sources in an Easter Island population (Dudgeon & Tromp, 2012)

Samples of dental calculus were first decalcified to extract the embedded microfossils, which were then studied using light microscopy from the extinct ape *Gigantopithecus blacki*, in order to identify the presence of embedded phytoliths (Ciochon, Piperno, & Thompson, 1990). This was followed by Middleton & Rovner (1994), who identified phytoliths and starch granules from samples of herbivores. Subsequently, starch granules (Juan-Tresserras, Lalueza, Albert, & Calvo, 1997; Scott Cummings & Magennis, 1997) and phytoliths (Lalueza Fox, Juan, & Albert, 1996) were extracted from human samples while a range of phytoliths was recovered from a sample of mastodon calculus (Gobetz & Bozarth, 2001). Capasso, Di Tota, Jones, and Tuniz (1995) evaluated the utility of synchrotron radiation microprobe analysis with potentially promising results, though this method has yet to be developed further. While many of these early publications highlighted the potential for extracting useful paleodietary information from dental calculus, none attempted to identify the specific sources of the microfossils.

Since 2008, the analysis of material remains entrapped in dental calculus has increased significantly. While this has largely focused on the extraction and identification of microfossils, in particular starch granules and phytoliths which emerge from the dental calculus matrix as it is decalcificed, a range of morphological and analytical methods are now used to access the material entrapped in the calculus (Hardy et al., 2012; Power, Salazar-García, Wittig, & Henry, 2014; Warinner, Rodrigues, et al., 2014; Warinner, Hendy, et al., 2014). However, the largest number of publications describes microfossil extraction and identification using optical microscopy. Most studies have focused on the identification of starch granules, and to a lesser extent phytoliths (e.g., Henry, Brooks, & Piperno, 2014; Henry & Piperno, 2008; Henry et al., 2012; Horrocks, Nieuwoudt, Kinaston, Buckley, & Bedford, 2013; Li et al., 2010; Mercader, 2009; Mickleburgh & Pagán-Jiménez, 2012; Piperno & Dillehay, 2008; Tao et al., 2015; Wang, Fuller, Wei, Chang, & Hu, 2015; Wesolowski, Ferraz Mendonça de Souza, Reinhard, & Ceccantini, 2010). The principal aim of these studies has been to identify dietary components, though food processing through evaluation of starch granule alteration has also been explored (Henry, Brooks, & Piperno, 2011; Henry, Hudson, & Piperno, 2009). However, the complexity of starch granule alteration has been highlighted, and suggests that further work is required to explore potential diagenetic processes (Barton & Torrence, 2015; Collins & Copeland, 2011).

Since 2015, an increasing number of papers have highlighted problems regarding the uncritical identification and interpretation of the data, most notably in terms of the integrity of the link between diet and the microfossils found, and their identification (Buck, Berbesque, Wood, & Stringer, 2015; Buck & Stringer, 2014; Leonard et al., 2015; Power et al., 2015; Radini, Buckley, et al., 2016; Wang et al., 2015). It has also become clear that accumulation of debris in calculus is more random than previously thought, which suggests that sampling as a proxy for full reconstruction is problematic. In terms of starchy food, it is clear that starch granules present in dental calculus do not represent dietary breadth though they may be more reliable at the population level (Leonard et al., 2015) while Power et al (2015) also detected dietary correlations at the level of populations, using phytoliths, though not with the starch granules. This suggests that broad interpretations of diet based solely on microfossil material should be viewed with caution.

The morphological study of all archaeological plant-based materials is based on comparison with reference collections. In relation to this, the need for a better understanding of taphonomic processes affecting starch granule survival is as applicable to material extracted from dental calculus as it is to the analysis of other archaeological materials, such as residues extracted from stone tools (Barton & Torrence, 2015). Likewise, although the preservation of starch granules is not in itself problematic due to the integrity of the mineralized matrix of calculus through time, the overlapping size and shape of starch granules that occur among and between species of plants, even in terms of widely used domesticated plants such as millets (Lucarini, Radini, Barton, & Barker, 2016; Wang et al., 2015), means use of starch granule morphology alone for detailed species identification is also problematic.

A range of analytical techniques have been used, often in combination with microscopy, to further characterize entrapped material. A study that combined scanning electron microscopy (SEM) with elemental analysis using energy-dispersive X-ray spectroscopy (EDX) has shown potential in detecting food and micro-debris linked to occupational habits (e.g., Charlier et al., 2010); however, it does not recover and identify as many particles as light microscopy (Power et al., 2014). Inductively coupled plasma mass spectrometry (ICP-MS) analysis of trace elements in dental calculus samples also seems promising and it has so far been used in the identification of carbohydrates and fish (Lazzati et al., 2016).

Stable isotope analysis has also been investigated for its potential to study the composition of materials embedded in dental calculus as a proxy for ancient dietary reconstruction (Scott & Poulson, 2012). However, Salazar-García, Richards, Nehlich, and Henry (2014) have demonstrated that the isotopic values obtained from dental calculus are not equal to those from bone collagen, possibly due to the diversity of material entrapped in the calculus and the variable amounts of calculus accumulation in individuals. This means the results obtained do not reflect a life-time average, as is the case with bone collagen. However, Wang et al. (2015) correlated carbon isotope data taken from samples of bone collagen, with starch granules extracted from dental calculus.

Thermal desorption–gas chromatography–mass spectrometry (TD–GC–MS) and pyrolysis–gas chromatography–mass spectrometry (Py–GC–MS) can be used to characterise a wide range of organic materials in dental calculus. The mineral component of dental calculus traps organic compounds and provides them with a protective environment, which allows biological marker compounds (biomarkers) characteristic of the original source to survive. The identification of specific chemical compounds enabled Hardy et al. (2012) to detect exposure of

Neanderthal individuals from the site of El Sidrón, Spain, to wood smoke, bitumen or oil shale and a range of plant items providing the earliest direct evidence for the use of medicinal plants in human history. Ingestion of the plant *Cyperus rotundus* L., was detected throughout the multi period sequence at Al Khiday, Sudan (Buckley et al., 2014). Today, this plant is considered primarily as a problematic weed in many countries (Sivapalan, 2013) suggesting that dental calculus has the potential to recover evidence for foods and medicinal plants that have been forgotten. The earliest direct evidence for plant foods in the genus *Homo* has been identified at the 1.2 million year old site of Sima del Elefante, Atapuerca, Spain (Hardy et al., 2016) while chemical evidence for plant-based nutrients was identified at the 300,000–400,000 years old Lower Palaeolithic site of Qesem Cave, Israel (Hardy et al., 2015).

Dental calculus is also a source of bacterial genetic material and holds potential as a biomolecular reservoir (Preus, Marvik, Selvig, & Bennike, 2011). A number of papers have reviewed the recent application of biomolecular techniques to the study of ancient dental calculus and its relevance to ancient diet and oral and overall health (Huynh, Verneau, Levasseur, Drancourt, & Aboudharam, 2016; Metcalf, Ursell, & Knight, 2014; Pacey, 2014; Weyrich, Dobney, & Cooper, 2015). De la Fuente, Flores, and Moraga (2012) amplified DNA and identified bacteria from 4000-year-old samples from Chile. Similarly, Adler et al. (2013) identified a wide range of oral bacteria, demonstrating a shift in their composition as carbohydrates from domesticated plants became prominent. Warinner, Rodrigues, et al. (2014) applied shotgun DNA sequencing to samples of historic age dental calculus. This study highlighted the link between oral pathogens, host immunity and dietary patterns by identifying in the oral environment, opportunistic pathogens, antibiotic resistant genes, bacterial and human proteins, as well as DNA sequences from dietary sources. Only a portion of the material identified using biomolecular evidence has been securely linked to diet (e.g., Warinner, Hendy, et al., 2014). However, much of this evidence, such as consumption of leafy crops of the Brassicaceae family traditionally have a low archaeological visibility (Warinner, Rodrigues, et al., 2014; Warinner, Hendy, et al., 2014). Warinner et al. (2015) also detected the first direct evidence of milk consumption by identifying the protein β-lactoglobulin (BLG) in human dental calculus from the Bronze Age (ca. 3000 BCE).

The use of biomolecular techniques may be limited in some cases, such as for the earlier stages of the Palaeolithic, due to the apparent degradation of biomolecular evidence in the deep past (e.g., Hardy et al., 2016), but they appear to hold great potential for more recent samples.

4 | MICROPARTICLES IN DENTAL CALCULUS AS A RESULT OF NON-DELIBERATE CONSUMPTION

There is little doubt that much of the material extracted from dental calculus represents items that found their way into the mouth during life due to deliberate ingestion. However, the human mouth is a recipient of countless other particles and debris, and dental calculus has the potential to trap any material that reaches the mouth by incorporating and embedding it into its mineralised matrix.

4.1 | Gastrophagy

Buck et al. (2015) suggest gastrophagy, or the eating of the stomach contents of an animal, may confound palaeodietary reconstruction. In this respect they suggest that chemical compounds indicative of yarrow and camomile found in the dental calculus of the El Sidrón Neanderthals (Hardy et al., 2012) were the outcome of the eating of the stomach contents of an animal that had previously eaten these plants rather than the result of deliberate ingestion of non-nutritious plants for self-medication (Buck et al., 2015). In fact, herbivores graze on a wide variety of different plants and often actively avoid these strong tasting plants (Hardy, Buckley, & Huffman, 2016). There is however, extensive evidence for chyme consumption among recent and modern hunter-gatherers who tend to make use of all the parts of killed animals (Sinclair, 1953) and its use among prehistoric hunter-gatherers has already been hypothesised (Speth, 2010). Chyme can provide valuable nutrients, in terms of partially digested plant materials; for example among the Inuit it provided access to lichens that had been made edible through partial digestion, and which formed an essential part of their diet particularly in winter when there was a lack of other plant-based resources (Sinclair, 1953). As phytoliths are silica bodies, and therefore are reasonably resistant to degradation, it is possible that they could endure through the partial digestion process that forms chyme, and then become embedded in dental calculus (Henry et al., 2014). For example, phytoliths have been found in samples of coprolites (e.g., Horrocks, Irwin, McGlone, Nichol, & Williams, 2003).

4.2 | Secondary eating of debris embedded or settled on food or drinks

Dust and dirt, including grit particles settled on food, can reach the mouth accidentally and become embedded in the dental calculus matrix (Blatt et al., 2011; Buckley et al., 2014; Charlier et al., 2010; Hardy et al., 2015, 2016). Soil particles can also be accidentally ingested on food. A good example of this can be found in Dudgeon and Tromp (2012) who retrieved large quantities of globular echinate palm phytoliths (found in many parts of palm plants, including fruits, fruit stones but also leaves) together with evidence for sweet potato tubers from samples of dental calculus taken from Rapa Nui. However, no palm species that produced such phytoliths were present in the location at the time the studied individuals lived (Tromp & Dudgeon, 2015) and it later emerged that the palm phytoliths may have survived in soil and became embedded in the skin of the edible tubers as they grew (Tromp & Dudgeon, 2015). This study illustrates the potential pitfalls of assuming that materials found in dental calculus are automatically the result of deliberate ingestion of food items or diet.

4.3 | The human mouth as a "dust trap": pathways of inclusion by inhalation

In humans, as in all other land animals, air inhalation occurs constantly. Breathing through the mouth occurs during speaking, eating, communicating, panting after exertion and when the nose is blocked by nasal mucus. It is safe to assume that many prehistoric and historic populations were exposed to high levels of smoke and soot, thus organic and inorganic "dust" and particles could have become embedded in dental calculus. Dust, which can be caused naturally by wind or generated by human activity, can produce high concentrations of breathable debris. Se, Mei, Inthavong, and Tu (2010) demonstrated that particles up to 70 μm are habitually inhaled through the mouth while larger particles can also be inhaled under certain conditions. The production of dust, often incorporating large amounts of particulate matter below 100 μm, is characteristic of a great variety of traditional human industries including stone-working, pottery manufacture, grain storage, certain types of food production such as grinding and cooking with flour and wood working. Such debris is considered a health hazard in modern societies, since particles below 10 μm can reach the lungs (Pope & Dockery, 2006). Respiratory health and air quality in past societies have received little or no attention so far, in part due to the paucity of evidence (Brimblecombe, 2011); however, exposure to smoke and pollutants is likely to extend deep into the human past (Makra, 2014; Naeher et al., 2007; Hardy et al., 2015, 2016). Histological assessment of the lungs of the Tyrolean Ice Man (Oetzi) which dates to 5400 cal. BP, as well as ancient human mummies (Egyptian, Peruvian, and Aleutian), has shown that anthracosis (a pathology of the lungs associated with prolonged exposure to smoky environments), has been a regular disorder since ancient times (Mirsadraee, 2014; Zimmerman et al., 1981). In this respect, a wide range of mineral and organic "dust" from materials such as traditional pigments are also known (Murr, 2009).

Examples of environmental material found in dental calculus, and most likely the result of inhalation, include insect parts, pollen, micro-charcoal, soot (which most likely represents smoke inhalation), minute grit and dust particles, plant fibers and phytoliths potentially derived from cultural practices (Blatt et al., 2011; Buckley et al., 2014; Hardy et al., 2015, 2016; Radini, Buckley, et al., 2016), or from accidental ingestion, for example, from bedding during sleep. An example of accidental inhalation/ingestion are the fungal debris such as spores and hyphae, which have been found in several studies (e.g., Hardy et al., 2015, 2016; Radini, Nikita, et al., 2016; Warinner, Hendy, et al., 2014). Fungal spores detected in dental calculus samples have been considered as food items (Afonso-Vargas, La Serna-Ramos, & Arnay-de-la-Rosa, 2015; Power et al., 2015). Hardy et al. (2015) highlight the ubiquity of fungal spores in the environment, and suggest that they may well result from accidental ingestion or inhalation. The fruiting bodies of fungal spores may become useful food items such as mushrooms; however, assuming that fungal spores in calculus are the result of deliberately ingested mushrooms needs to be viewed with caution.

4.4 | The role of extramasticatory uses of teeth

A further source of non-dietary debris in the mouth is the use of teeth in non-masticatory activities (e.g., Blatt et al., 2011). Physical evidence for the non-masticatory use of teeth, including tooth wear is extremely common in prehistoric, historic and some modern human populations from across the world (Bonfiglioli, Mariotti, Facchini, Belcastro, & Condemi, 2004; Eshed, Gopher, & Hershkovitz, 2006; Hinton, 1981; Lozano, Mosquera, de Castro, Arsuaga, & Carbonell, 2009; Lukacs & Pastor, 1988; Molnar, 2008; Ryan & Johanson, 1989; Volpato et al., 2012). The use of teeth to hold, soften or shred material, as well as in grooming activities and leaf chewing is widespread in the ethnographic record (Hardy, 2008, 2016). One example comprises an Inuit population with extreme dental wear among the anterior teeth, which was linked to the preparation of walrus and seal hides for clothing, making threads out of animal sinew (Clement & Hillson, 2012; Mayhall, 1976; Pedersen, 1947). Non-dietary chewing of leaves, such as tobacco or betel nut chewing in humans, or leaf-rolling in the mouth of chimpanzees (e.g., McLennan & Huffman, 2012) could explain the presence of calcium oxalate in the dental calculus of primates found in recent studies (Power et al., 2014).

4.5 | Food preparation, food stickiness, oral clearance, and oral hygiene

Food preparation practices can significantly alter the structure and nutritious quality of plants (Butterworth, Ellis, & Wollstonecroft, 2016) and can also have important effects on dental calculus in terms of the formation and survival of micro-debris. For example, starch granules and phytoliths can be altered and degraded by the use of grinding tools in food preparation (e.g., Lucarini et al., 2016). The use of grinding tools can incorporate grit and stone particles into the food which accelerate tooth wear and can also lead to particles becoming embedded in dental calculus. In addition chewing starchy food, even when uncooked, frees the starch granules from their enclosing amyloplasts enhancing the effects of amylase in the saliva, which results in the further breakdown of these granules (BeMiller & Whistler, 2009).

The way food preparation practices may affect the formation of calculus has been given little consideration. As already discussed, chewing abrasive materials can mechanically remove calculus deposits (Gaar et al., 1989) but the way food is prepared and its relative softness affects dental calculus build up just as it affects the rate of dental wear. Several studies have demonstrated the association between food processing and dental wear levels, whereby processed soft-textured food with low fiber content results in less dental wear (Deter, 2009; Eshed et al., 2006; Molnar, 1971). Alternatively, the ingestion of sand, grit and other hard particles that become embedded in the food during grinding, drying and other food processing practices, results in increased dental wear (e.g., Lev-Tov Chattah & Smith, 2006; Smith, 1984). However, the correlation between dental calculus build-up and preservation and dental wear as a proxy for food processing practices, has yet to be explored.

In addition to its potential effect on dental calculus preservation, cooking tends to alter the properties of various foodstuffs and other inclusions, rendering many of them often impossible to identify in dental calculus deposits. Proteins become denatured and various food borne pathogens are killed (Carmody & Wrangham, 2009; Gaman & Sherrington, 1996; Radley, 1968; Svihus, Uhlen, & Harstad, 2005; Tester, Qi, & Karkalas, 2006). The effect of cooking on starch granules is well established, and results in their disruption and gelatinisation at which point they are no longer visibly present as microfossils in dental calculus, though a small amount of starch granules are resistant to degradation and can survive (Radley, 1968). In some cases, particularly when cooked using traditional open cooking methods, some food does not "cook through." In this case, not all starch granules disrupt and can survive intact after having been cooked using outside ovens (Buckley et al., 2014; Schnorr, Crittenden, & Henry, 2016; Thoms, Laurence, Short, & Kamiya, 2015).

The roles of food stickiness, salivary clearance and oral hygiene are also relevant when evaluating potential for diet on the basis of microfossils entrapped in calculus, because such aspects may affect the quantity of dental calculus build up as well as the typology of debris. Food stickiness refers to the ability of certain foods to adhere to dental and other oral surfaces (Lucas, Prinz, Agrawal, & Bruce, 2004). Many factors determine stickiness, including water content in the food, its viscosity, as well the nature of the food ingredients (e.g., sugars of low molecular weight generate increased stickiness, whereas carbohydrates and proteins of high molecular weight tend to minimize stickiness, see review in Adhikari, Howes, Bhandari, & Truong, 2001). Food stickiness is an important aspect to be taken into consideration during dental calculus studies as particles of stickier food may well be more likely to become embedded in dental calculus, though further work is required to investigate the implications of this in terms of dietary reconstructions using dental calculus.

Clearance from the oral cavity is one of the most important salivary functions since it removes food and dirt and maintains correct pH and oral biofilm in the oral cavity (Humphrey & Williamson, 2001). Different food types (sugars and fat) clear at different rates, though no direct link between food stickiness and speed of clearance has been detected (Dawes et al., 2015). The extent to which food-related microdebris can become incorporated into the calculus matrix is likely to depend on how long and how much the food and other debris stick to the tooth and how fast each category of ingested debris takes to be swallowed and removed from the mouth. Therefore the nature of food preparation may further affect the archaeological record entrapped in the calculus matrix, though further work is needed to address this.

The property of food stickiness, as well as fibrous debris that can become entrapped between teeth, generate the need for oral hygiene practices. Dental hygiene is neither a product of modern society, nor an exclusively human phenomenon. Chimpanzees have been recorded cleaning each-other's teeth using their fingers and sticks (McGrew & Tutin, 1973). Japanese macaques use their hair or the hair of another individual to floss between their teeth (Leca, Gunst, & Huffman, 2010), while long-tailed macaques in Thailand which interact intensively with humans, have been observed to use human hair as flossing material (Watanabe, Urasopon, & Malaivijitnond, 2007).

Among prehistoric human populations, a well-known case proposed as evidence for oral hygiene activities are the interproximal grooves on hominin teeth (Brothwell, 1963) for which tooth picking has long been suggested as a cause (Ubelaker, Phenice, & Bass, 1969). Interproximal grooving has been found on samples of all hominin species since *Homo habilis* (Puech & Gianfarani, 1988) and the grooves are widespread among Neanderthal populations (Dąbrowski et al., 2013; Estalrrich et al., 2011; Formicola, 1988; Frayer & Russell, 1987; Lozano, de Castro, Carbonell, & Arsuaga, 2008; Urbanowski et al., 2010; Villa & Giacobini,1995). Many materials have been suggested as tooth picks including wood, and grass (Brown & Molnar, 1990; Eckhardt & Piermarini, 1988; Hlusko, 2003). While a number of hypotheses have been put forward that attempt to explain interproximal grooves, tooth picking to extract food stuck in between teeth is still considered to be the most likely cause (Ungar, Grine, Teaford, & Pérez-Pérez, 2001). Grass contains abundant phytoliths and it has been suggested that with prolonged use, these are sufficiently abrasive to cause the grooves (Hlusko, 2003).

The use of chewing sticks to maintain oral hygiene is widespread in traditional societies (Almas, 2002; Idu, Umweni, Odaro, & Ojelede, 2009; Jose, Sharma, Shantaram, & Ahmed, 2011). Many of the plants used for chewing have antibacterial qualities, which may aid oral hygiene (Idu et al., 2009; Jose et al., 2011). In a Dakshina Kannada population from India for example, 25 different plants are used in oral hygiene, either being chewed, or applied to the mouth. Each plant has a specific role, including treatment of oral ulcers, gum disease, tooth decay, toothache, caries and stomatitis. Other materials, including charcoal, soot, clove oil, ghee, honey, and salt are also used to clean teeth and relieve pain (Jose et al., 2011). All of these could potentially leave chemical traces or microfossils embedded in dental calculus. Likewise, oral hygiene practices are likely to have had an impact on the preservation of dental calculus, as they may remove or reshape deposits. The possibility that plant parts and grass were used in oral hygiene suggests that phytoliths found in dental calculus need to be viewed with an open mind as to their origin, and that reference collections need to be sufficiently broad to incorporate other potential sources, in addition to food. Finally, oral hygiene may be responsible for the removal or remodelling of dental plaque during life therefore reconstructions of diet need to be conducted with great caution as the extent of a person's life that is reflected in the calculus deposit is unknown. Two recent dental calculus studies have noted the presence of non-edible wood fibers with interproximal grooves; in one case Radini, Buckley, et al. (2016) reported chemical compounds and microdebris derived from non-edible conifer wood fragments embedded in Neanderthal calculus samples adjacent to teeth with extensive tooth wear and morphological evidence for tooth picking from El Sidrón, Spain, while Hardy et al. (2016) also detected non-edible wood fiber from a sample of dental calculus from the 1.2 million year old Sima del Elefante site.

4.6 | Archaeological evidence of non-dietary remains in human dental calculus

A number of studies have demonstrated that non-dietary micro-debris is preserved in the calculus matrix of archaeological samples. Blatt et al. (2011) recovered evidence of cotton fibers (*Gossypium* sp.) in dental calculus samples from Danbury, Ohio, dated 900–1100 AD. Charcoal debris and mineral "grit" were found in the dental calculus of a Brazilian

Sambaqui population, though this was interpreted as possible contamination (Wesolowski et al., 2010). Charlier et al. (2010) found, in addition to probable food debris, plant fibers possibly generated by the use of the mouth as a third hand, as well as some mineral debris/crystals, possibly from sand and grinding stones, which may have been either inhaled or ingested. Elemental analysis of some of the crystals trapped in dental calculus identified debris derived from the environment in several individuals and possibly work-related pollution in one individual from the Etruscan population of Monterenzio Vecchia (Charlier et al., 2010). Hardy et al. (2012) identified evidence for smoke inhalation and bitumen which may be related to tool hafting. Buckley et al. (2014) identified microcharcoal and numerous plant fibers that suggest the non-masticatory use of the teeth in a prehistoric Sudanese population with extensive non-masticatory dental wear, while Hardy et al. (2015) retrieved evidence of exposure to potential respiratory irritants in samples of Lower Palaeolithic hominins. Radini, Nikita, et al. (2016) also found evidence of plant fibers consistent with flax and/or hemp in dental calculus samples from Medieval Leicester (UK).

Consideration of all of these lines of evidence points to a degree of complexity and multiple potential pathways by which material can become entrapped in human dental calculus. It is not currently clear whether the limited published information on non-dietary debris is the result of their absence in the samples, or because this debris has not been identified or reported.

5 | CONCLUSIONS

The research on ancient human dental calculus has significantly increased, as the potential for reconstructing aspects of diet has become apparent. However, while the potential of dental calculus to entrap micro-remains of dietary origin has been recognized, the link between such debris and the deliberate consumption of plants by ancient people and their role in diet is more complex than previously thought. Through a number of pathways, such as accidental ingestion, oral breathing and the use of the teeth as a third hand, the human mouth experiences a continued ingress of organic and inorganic particles that are present in the environment or derived from human activities. Though the many ways debris can become embedded in dental calculus may limit its usefulness in the detailed reconstruction of ancient diet, they increase its value for obtaining wider biographical information on past individuals. The approach of combining microscopy with various analytical techniques and osteoarchaeological parameters can help to differentiate between dietary and non-dietary debris (Radini, Buckley, et al., 2016). Likewise, given the presence of material that is not related to diet, reference collections, which form the basis of all identification of material remains, should incorporate non-dietary plants and plant parts, including those used in material culture and oral hygiene, as well as atmospheric and environmental contaminants.

One of the problems in understanding the sources of the material embedded in dental calculus is the difficulty in conducting experimental or modern comparative work. The use of live traditional populations in this respect can potentially be problematic as it requires invasive research techniques and may encourage or support calculus build up, which is detrimental to health. Therefore ethics must be taken in account when working with live human populations, though experimental archeology may help to clarify the flux of particles in the mouth. Likewise, more research on recent archaeological populations for which there is greater information available, will help to enhance our understanding of the extent to which dental calculus is representative of the daily life of the studied individuals (Radini, Nikita, et al., 2016).

The study of dental calculus has come a long way since its potential for informing on past human and hominin lives was first recognised. The quality and value of information that can be obtained from the extraction and identification of embedded microfossil material, and the recovery and identification of *all* microfossil material, covers a much wider range of information than simply diet. Recovery of all material requires very gentle decalcification methods while identification of the material can be challenging. Likewise, to maximise this information and identify material that is unequivocally linked to diet, the material remains also need to be contextualised within a broader framework. Conducted in this way, the analysis of microfossils extracted from dental calculus and the recognition of their potential to provide environmental and cultural, as well as dietary information will ensure this relatively new subdiscipline develops to provide a wide range of exciting data and information on past lives and environments, within realistic parameters.

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