

# Dental calculus – oral health, forensic studies and archaeology: a review

Roger Forshaw<sup>1</sup>

## Key points

Provides an overview of dental calculus from the viewpoint of the dental surgeon, the forensic specialist and the archaeologist.

Dental calculus, being among the richest biomolecular source in the archaeological record, is able to provide significant new insights and answers to questions, relevant to both archaeology and anthropology.

Considers how dental calculus analyses can be useful in shedding light on ancient diet, disease and lifestyle patterns.

## Abstract

Dental calculus is recognised as a secondary aetiological factor in periodontal disease, and being a prominent plaque retentive factor, it is routinely removed by the dental team to maintain oral health. Conversely, dental calculus can potentially be useful in forensic studies by supplying data that may be helpful in the identification of human remains and assist in determining the cause of death. During the last few decades, dental calculus has been increasingly recognised as an informative tool to understand ancient diet and health. As an archaeological deposit, it may contain non-dietary debris which permits the exploration of human behaviour and activities. While optical and scanning electron microscopy were the original analytical methods utilised to study microparticles entrapped within the calcified matrix, more recently, molecular approaches, including ancient DNA (aDNA) and protein analyses, have been applied. Oral bacteria, a major component of calculus, is the primary target of these aDNA studies. Such analyses can detect changes in the oral microbiota, including those that have reflected the shift from agriculture to industrialisation, as well as identifying markers for various systemic diseases.

## Introduction

Dental calculus can be defined as a complex mineralised plaque biofilm which is sequentially generated and entraps microbial, dietary, host and ancient debris during spontaneous calcification events.<sup>1,2</sup> It is composed primarily of calcium phosphate mineral salts deposited between and within remnants of formerly viable microorganisms and is covered by an unmineralised bacterial layer.<sup>3</sup>

Dental calculus forms throughout an individual's life on the subgingival and/or supragingival tooth surfaces. There is a cohesive bond between crystals in the calculus and the enamel, dentine or cementum apatite crystals at the calculus-tooth interface.<sup>4</sup> Calculus is found in all known populations, past and present, but

the extent varies widely among individuals and populations. Factors such as oral hygiene routine, frequency of dental care, age, systemic health, diet and ethnicity all affect its formation.

Dental calculus has long been recognised as a significant factor in the aetiology of periodontal disease. It is a rich source of human DNA and as such, has the potential to serve as an investigative tool in forensic studies. As calculus is mineralised, it often survives well in archaeological contexts and is useful when studying the dental pathology of our ancestors. It is only comparatively recently that the vast research potential of dental calculus for archaeological study has been appreciated, providing as it does insights into ancient diet, health, disease and evolutionary history. This present study aims to review dental calculus in relation to these three topics of oral health, forensic investigations and archaeological research.

## Dental significance

Epidemiological studies have long demonstrated a strong association between calculus and periodontal disease.<sup>1,5,6</sup> Dental calculus does not contribute directly to gingival irritation but it provides a nidus for the continued

accumulation of plaque. It retains plaque in close proximity to the gingivae and creates areas where plaque removal is difficult or impossible, all contributing to gingival inflammation and periodontal disease.<sup>7,8</sup> Calculus provides an ideal porous vehicle for bacterial plaque retention and growth and is therefore regarded as a secondary aetiological factor in periodontal disease.

Once formed, the presence of calculus may compromise oral hygiene procedures and promote the growth of pathogenic plaque. As a prominent plaque retentive factor, the deposits must therefore be removed for adequate periodontal therapy and the maintenance of oral health.<sup>3</sup> The removal of plaque and calculus is the basis of periodontal treatment and the dental team expends considerable effort and resources on this aspect of modern-day dental care.

There are wider health implications for the build-up of calculus deposits. Individuals with a relatively high disease susceptibility and who have poor oral hygiene and calculus deposits that have resulted in marked destruction of the periodontal tissues are more at risk of systemic disease.<sup>9</sup> Periodontal disease may predispose the patient to an increased incidence of bacteraemia which can cause infective endocarditis, a condition that ultimately may be fatal.<sup>10</sup> There is a link between

<sup>1</sup>KNH Centre for Biomedical Egyptology, Faculty of Biology, Medicine and Health, Manchester University, Stopford Building, Oxford Road, Manchester, M13 9PL, UK.  
Correspondence to: Roger Forshaw  
Email address: Roger.Forshaw@manchester.ac.uk

Refereed Paper.

Submitted 28 January 2022

Revised 25 April 2022

Accepted 9 May 2022

<https://doi.org/10.1038/s41415-022-5266-7>

chronic inflammation of the periodontal tissues and the risk of the affected cells of the oral epithelium becoming malignant.<sup>11</sup> Also, there is a two-way relationship between diabetes and periodontal disease. Diabetes increases the risk of developing periodontal disease and periodontal inflammation adversely affects glycaemic control.<sup>12</sup>

After removal from the teeth of patients, calculus debris is routinely disposed of, as being a clinical waste product, it is of little use. However, in recent years, dental calculus has been suggested as having a possible role in forensic investigations and accepted as a tool in helping to understand ancient biographical and dietary information.

## Forensic studies

Analysing genetic material is the most accurate method for establishing human identity in forensic examinations. Where human remains are degraded or fragmented, teeth and bones may be the only sources of DNA available for identification. The unique composition of teeth and their locations in the mandible and maxilla provide additional protection to DNA compared to bones, making them a preferred source of DNA in many cases.<sup>13</sup> The dental pulp is the usual source of DNA but where there is poor preservation of the human remains or where permission for destructive sampling is denied, then accessing this source can be problematic.

However, previous studies have reported that DNA is present in dental calculus (see the human DNA analysis section) and so the biofilm can provide an alternative source. A pilot study has been able to demonstrate the potential of

dental calculus to serve as an investigative tool in forensic studies.<sup>14</sup>

Charlier and his co-workers<sup>15</sup> investigated archaeological samples of calculus of individuals from a number of historic sites, utilising microscopy coupled with elemental surface analysis. Their results provided information about food, environmental habits and work-related exposure to pollutants. Such data can be helpful in determining individual habits and pathologies, potentially useful in identification and determining cause of death.

Recently, a new, sensitive, ultra-high-performance, liquid chromatography-tandem mass spectrometry technique has been developed, a method that is capable of demonstrating a large variety of pharmaceutical and psychoactive drugs entrapped and preserved within calculus. Such inclusions would have been derived from direct contact with sources in the oral cavity, from inhalation of smoke or vapour and from the release of serum into the saliva and gingival crevicular fluids. Many drugs, including heroin, cocaine and opium, are able to be identified by this method – drugs that may not have been originally detected in the blood at the time of autopsy but which may have been a factor in the cause of death. This technique is not only applicable to modern forensic investigations but can also provide an overview of drugs and stimulants used in ancient time periods.<sup>16</sup>

## Drug entrapment in calculus

Archaeological evidence has demonstrated that ancient peoples used plant-based remedies for the treatment of medical conditions. Originally, most of the information about these

preparations was derived from the material culture, osteological evidence of pathologies and surgical interventions, archaeobotanical analyses and textual sources. These data are now supported by the detection, in calculus, of plants now known for their medicinal properties.<sup>17,18,19</sup>

The wide and varied range of plants that have been identified in the dental calculus of Neanderthals would suggest that they had a sophisticated understanding of their environment. Certain bitter-tasting plants, with no nutritional value, containing compounds such as yarrow and camomile, suggest that they had the ability to select and use particular flora for medicinal purposes.<sup>17</sup>

Dental calculus analyses of an early medieval Italian population of Colonna in central Italy, dating back to the eighth to the tenth century AD, also demonstrated similar results. Not only was a detailed qualitative reconstruction of the food habits of this community able to be obtained, but the identification of specific chemical indicators suggested the pharmaceutical application of a number of medicinal plants. Among these were: *Digitalis* sp., used in treatment for heart conditions; *Hyssopus officinalis*, recognised for its antiseptic and expectorant properties; *Artemisia* sp., having digestive, antiseptic, antimalarial and expectorant capabilities; and *Ephedra* sp., a bronchodilator and vasoconstrictor.<sup>19</sup>

Again, dietary intake and plant-based treatments were studied in a woman buried in the Late Preceramic site of Huaca El Paraíso, Peru (2100–1500 BC). In this case, an analysis of the calculus was supplemented by examining the sediment enclosed inside her grossly carious mandibular molars (Figures 1 and 2).<sup>20</sup>



**Fig. 1** a, b, c) Selected phytoliths and starch grains identified in dental calculus samples from a woman from the Late Preceramic period, Peru. Micrographs taken at x400 magnification. Reprinted from *Annals of Anatomy*, Vol 240, Allende et al., 'Dental anthropological report: Exploring plant-based treatments through the analysis of dental calculus and sediment of dental caries in a woman from the Late Preceramic period, Peru', 2022, with permission from Elsevier<sup>20</sup>

## Heavy metal poisoning

Heavy metal exposure has become a serious health concern in recent decades, particularly with the ubiquity of these elements in our daily environment. Various analytical methods, such as transmission electron microscopy, have been used in the assays of the major and minor elements found in supragingival dental calculus.<sup>21</sup>

Cadmium is a widespread-trace toxic heavy metal with a long biological half-life and is considered to induce a higher risk of cancer in multiple organs of the human body. A recent study has confirmed the relationship between cadmium levels in dental calculus, due to betel-quid chewing and smoking, with the subsequent risk of oral cancer.<sup>22</sup> Tobacco smoke is a complex and reactive mixture of numerous chemicals that include a number of heavy metals. Again, as calculus is a biological material that can be collected non-invasively, it can be useful in monitoring oral heavy metal exposure.<sup>23</sup>

Analysis of the calculus from the mandibular teeth of Agnès Sorel, mistress of the French King Charles VII, who was buried in Loches, France in 1450 AD, revealed a very high level of mercury, sufficient to have caused death by acute mercury poisoning. Evidence suggests that Agnes suffered from roundworms for which mercury was a common treatment at that time. Mercury was also used to treat women in labour for difficult deliveries and Agnes was known to have borne four daughters by the king. However, the evidence of a massive dose of this heavy metal hints at foul play and while it is not known if her death was deliberate or accidental, it has been suggested that her undue influence over the king would have created enemies for her at court.<sup>1,5,24</sup>

## COVID-19

The SARS-CoV-2 virus is the causal agent for COVID-19 and the high yield of the virus in salivary secretion is a common finding

in the infection.<sup>25</sup> As calculus contains biomolecules, such as nucleic acids, it has been hypothesised that following infection, SARS-CoV-2 ribonucleic acid could be preserved in calculus. A recent study in previously infected individuals has been able to confirm this proposition.<sup>23</sup> Samples of dental calculus were analysed by performing reverse transcription polymerase chain reaction assays following nucleic acid extraction and amplification. As well as in infected cases, this technique may also be able to detect traces in asymptomatic or mild asymptomatic patients. However, further studies are needed to define the method's reliability, cost-effectiveness and suitability for large scale epidemiological studies or post-mortem analyses.<sup>26</sup>

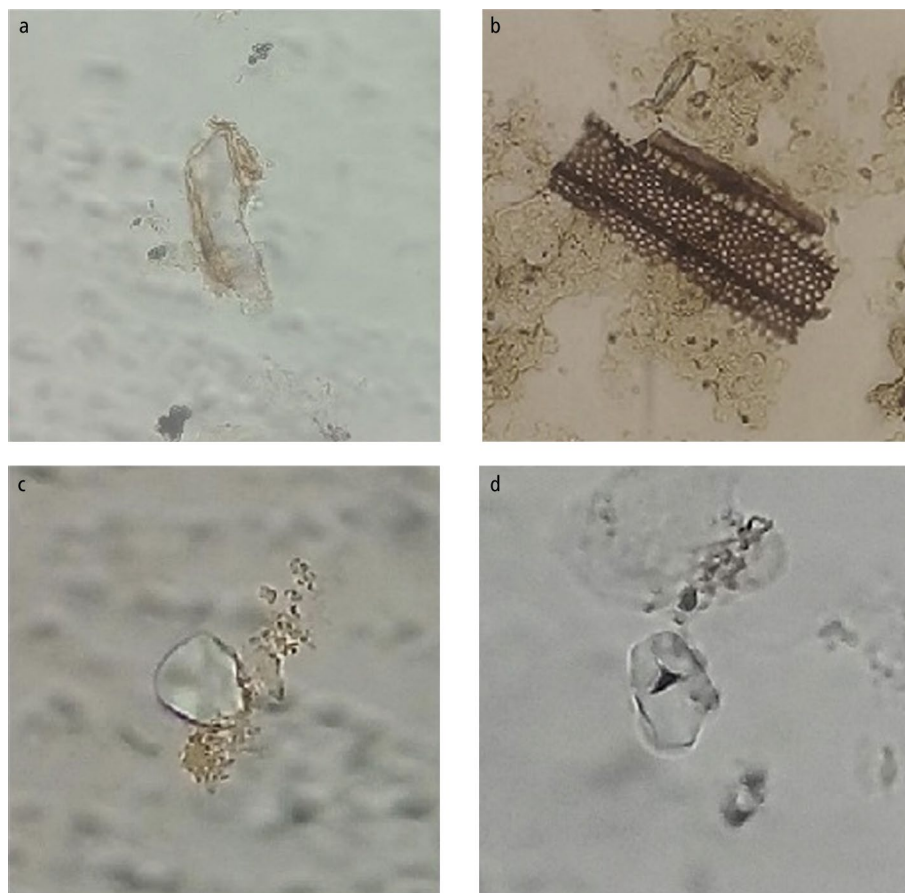
## Human DNA analysis

The bulk of DNA in dental calculus is microbial and originates from the oral microbiota (community of microorganisms within the oral cavity); however, a small, consistent and genetically rich proportion is endogenous human DNA. The mechanisms by which human DNA is incorporated into dental calculus are not fully understood. They are presumed to include passive adsorption of human DNA from oral fluids and shed mucosal cells, with more active incorporation through host inflammatory responses, such as an immune response mediated by neutrophils.<sup>2,27,28,29</sup>

Today, DNA technology has many applications, including studies into ancient DNA (aDNA), which can provide snapshots of the genetics of ancient populations. aDNA has revolutionised how we study many aspects of the biological past, including population origins and movements, natural selection, evolutionary relationships and identifying the presence of disease pathogens. As dental calculus is less porous and thus less susceptible to degradation by environmental microbes than dentine or bone, it offers an alternative source of ancient human DNA that may persist when other skeletal tissues fail to yield aDNA.

## Archaeological research into dental calculus

The study of dental calculus goes back to the 1980s when calculus deposits were partially decalcified and then viewed under an optical microscope. Supragingival calculus deposits are typically chosen as subgingival



**Fig. 2** a, b, c, d) Selected phytoliths identified in dental caries in a mandibular molar of a woman from the Late Preceramic period, Peru. Micrographs taken at x400 magnification. Reprinted from *Annals of Anatomy*, Vol 240, Allende *et al.*, 'Dental anthropological report: Exploring plant-based treatments through the analysis of dental calculus and sediment of dental caries in a woman from the Late Preceramic period, Peru', 2022, with permission from Elsevier<sup>20</sup>



calculus is more tightly adherent to the tooth surface, more heavily mineralised and is affected by haemorrhagic components from the gingival crevicular fluid. The deposits need to be carefully sampled, prepared and degraded with dilute hydrochloric following a standard protocol before microscopic examination.<sup>30,31,32,33</sup>

The technique is able to identify microparticles preserved within the matrix, which includes fragments of cereals, vegetable fibres, phytoliths, pollens, seeds, animal hairs, parasites and even insects that accidentally become entrapped (Figures 3 and 4).<sup>34,35</sup> Subsequent work examined the effects of ancient diets on calculus formation<sup>36</sup> and utilised calculus to study the diet and palaeoenvironment of Neanderthals and ancient humans.<sup>30,37,38</sup>

Far greater magnification can be achieved by the use of scanning electron microscopy (SEM), a technique often used as complementary with optical microscopy (OM). Calculus samples are mounted on stubs and sputter coated with gold to prevent surface charging by the electron



**Fig. 3** Medieval mandible showing calculus build-up. Site A24, 2003, Vine Street, Leicester, UK. Reproduced with permission from Anita Radini



**Fig. 4** A small invertebrate entrapped in dental calculus of a wild chimpanzee revealed in a scanning electron microscope image. Reproduced with permission from Robert Power and Heiko Temming

beam.<sup>31</sup> Elemental analysis of particles can be studied by using SEM with energy-dispersive x-ray spectroscopy.<sup>39</sup>

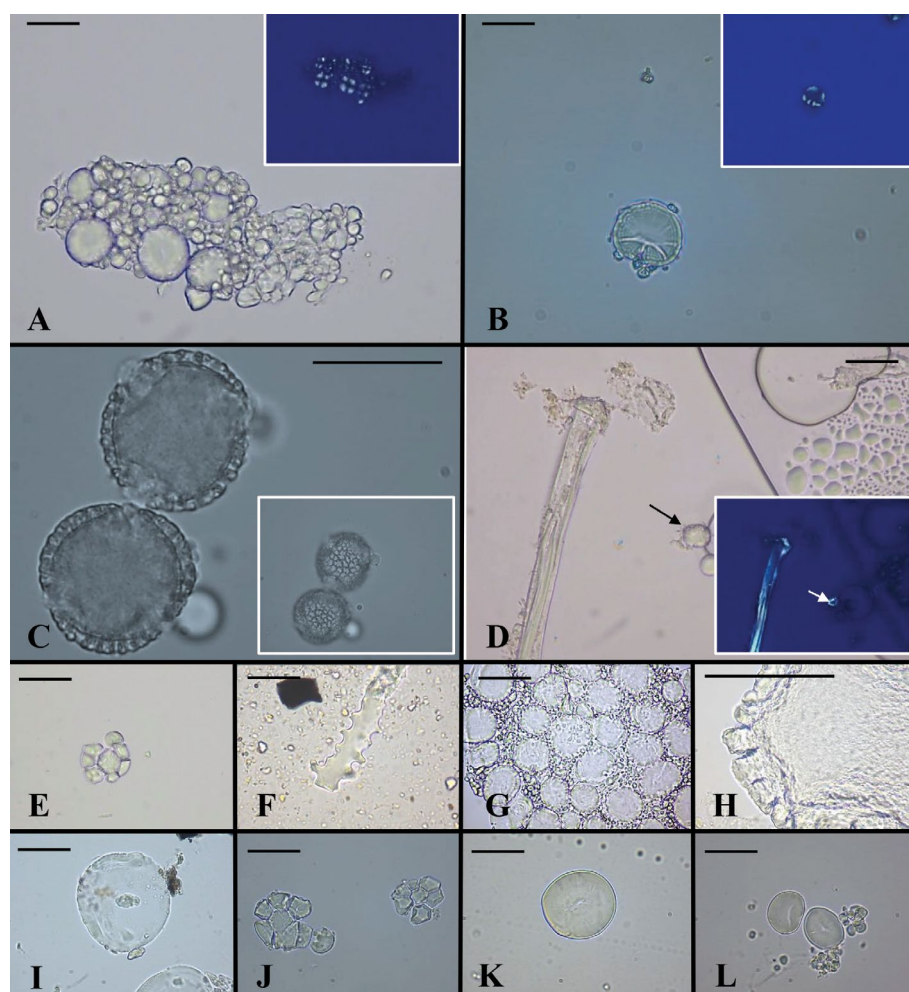
With the application of newer scientific methods in archaeology, bacterial DNA and host mitochondrial DNA preserved within the calculus are now successfully able to be extracted and analysed.<sup>40,41</sup> These continuing technological developments have radically improved the ability not only to extract DNA, but also proteins and metabolites. The research is reshaping our understanding of past diet, behaviour, ancestry, occupational activities and the health of past individuals and populations.

In biomolecular studies, aDNA and proteins are extracted from decalcified dental calculus, often utilising a unified protocol, which decreases the material needed for analyses while maximising the information yield, as described for example by Mackie *et al.*<sup>42</sup> and Fagernäs *et al.*<sup>43</sup>

## Dietary information

The study of plant microremains (phytoliths and starch grains) retained in dental calculus is a technique that is being increasingly utilised to determine ancient dietary information, as well as evidence about past environments and human cultures (Fig. 5). The analysis has the potential for revealing the genera and species of dietary plants, patterns of cultivation and methods of food preparation.<sup>37,44,45,46,47,48</sup> Additionally, it is a useful method for studying population-level dietary trends.<sup>49</sup>

Today, starch-based foods constitute 50–70% of the energy intake of most humans and they also had this important role in the pre-agricultural human diet.<sup>44</sup> The use of starches trapped inside dental calculus provides a direct link to food consumption and as the particles are confined inside a calcified matrix, they are less likely to alter over time.



**Fig. 5** Images of starch granules and other microremains entrapped within dental calculus utilising light microscopy. a, b, c, d, e, f, g, h, i) Archaeological samples. j, k, l) Modern starch references. The scale bar indicates 20 µm. Reprinted with permission from Gismondi *et al.*, 'Back to the roots: dental calculus analysis of the first documented case of coeliac disease', *Archaeological and Anthropological Sciences*, 2020, Springer Nature<sup>74</sup>

Calculus analysed from the Shanidar (Iraq) and Spy (Belgium) Neanderthals indicates that they consumed a considerable amount of plant materials. These included date palms, legumes and grass seeds, suggesting that their diet was not primarily based on meat as had previously been proposed. The range of local plant foods that they consumed was diverse, some of which they cooked, implying an overall sophistication in Neanderthal dietary regimes.<sup>38</sup>

A study of teeth from a middle Holocene (circa 5500–4500 BC) archaeological site in Syria was able to determine that the individuals were consuming a variety of plant foods. Domesticated cereals, such as wheat and barley, which the archaeological record had previously hypothesised as supplying the major sources of starches, was found to make up a surprisingly small portion of the diet.<sup>38,50</sup>

Researchers investigating a forager of the Central Mediterranean region, dating to the end of the eighth millennium BC (the Mesolithic Period), utilised a combination of dental calculus analysis and stable isotope techniques to reveal inclusions of fish scales, fish and bird flesh, starch granules and other plant and animal microdebris. The results indicated that marine resources, together with a variety of plant foods, were regularly consumed by the individual, whereas previous stable isotope data alone had indicated that it was mainly terrestrial-based resources that were the main contribution to Mesolithic diets.<sup>51</sup>

Similarly, data obtained from individuals of the older Magdalenian culture (17000–12000 BP) in Northern Iberia, showed that plant and plant-like foods were also parts of their diet. The results indicated a mixed subsistence economy, rather than the previously held supposition of a diet largely based on protein.<sup>52</sup>

Microfossil analysis has more recently been combined with proteomic (large scale study of proteins) and aDNA-based approaches to provide more accurate interpretations of entrapped dietary information within calculus. Microfossil analysis utilising OM and SEM allows morphological matches of entrapped calculus particles, which, when associated with bimolecular studies, provides complementary information that is able to characterise many dietary components at a higher taxonomic resolution.<sup>17,28</sup>

## Migrations and dietary shifts

Previous studies have indicated that aDNA derived from human skeletal remains can be used to determine historical human migrations

around the globe.<sup>53,54,55</sup> More recently, it has been established that an analysis of aDNA from microorganisms preserved within calculus also has the potential to reconstruct human migration and interaction networks. Tracking human migrations by this method involves the identification of genetic mutations within bacterial species. The composition of the human oral microbiota is relatively distinct to each culture and geographic region. This characteristic highlights the potential ability of microbiota DNA within dental calculus to provide an ancient genetic signal of cultural affinity, therefore advancing our understanding of human prehistory.<sup>56,57,58</sup>

These types of analyses can also identify changes in human oral microbiota communities that correlates with major dietary alterations over time. The transition from hunter-gatherer to farming shifted the oral microbial community to a disease-associated configuration, with a marked increase in the prevalence of dental calculus and oral pathology.<sup>59</sup> The composition of oral microbiota remained constant between Neolithic and medieval times, after which cariogenic bacteria became dominant during the Industrial Revolution, with the advent of industrially processed flour and sugar.<sup>57</sup>

## Prehistory

Reconstructing detailed aspects of the lives of our distant ancestors has always proved challenging due to the restricted nature of the surviving evidence. Poor preservation of plant remains and the lack of systematic recovery techniques for organic residues have prevented an understanding of dietary habits in pre-agrarian societies. However, the recovery of botanical microfossils and other debris from archaeological dental calculus has the potential to provide not only plant-related evidence, but also to contribute to the reconstruction of non-dietary related practices in everyday life. Such information is not always available in the archaeological record.

Dental calculus can be retrieved from skeletal material dating to most archaeological periods. Despite their extreme age, in some cases, the results from the analysis of the material can shed light on hitherto unknown aspects of ancient life. Excavations that were undertaken in Qesem cave in Israel has produced information relating to Lower Palaeolithic hominids who lived some 420000–200000 BP. The population were found to have consumed a very broad range of plant species, suggesting an ecological knowledge sufficiently developed to permit the selection of

physiologically essential plant foods. In addition, the presence of micro-charcoal fragments of up to 70 µm indicates a smoky atmosphere inside the cave and perhaps points to the ingestion of cooked or smoked food.<sup>32</sup>

Calculus samples from the multi-period (pre-Mesolithic to Meriotic) Central Sudanese site of Al Khiday has also produced evidence for cooking and smoke inhalation. Among the plant evidence was found samples of *Cyperus rotundus* (purple nut sedge), a perennial found to have been consumed during all periods. The non-nutritional qualities of the plant suggest that its use may have been directed at its aromatic or medicinal properties. *C. rotundus* is known to inhibit *Streptococcus mutans*, which is associated with the initiation of dental caries.<sup>60,61</sup> Chewing of *C. rotundus* tubers may have contributed to the unexpectedly low prevalence of caries in the Meriotic samples from Al Khiday.<sup>62</sup>

Calculus obtained from individuals buried at Nemrik 9, a Neolithic site in Northern Iraq, dating to 9100–8600 BP, was not only able to provide insights into diet at this early farming community, but was also able to provide some evidence that plants may have been used as tools. The shape and pattern of entrapped wooden splinters and phytoliths indicated that either wood and reeds may have been processed using teeth as an implement, or that these materials were used as tooth picks.<sup>63</sup>

## Stable isotope analysis

Stable isotope analysis of biomaterials, such as bone, teeth, fingernails and hair, has become a recognised technique for palaeodietary analysis in bioarchaeology.<sup>64,65</sup> The technique is destructive, as the sample material is destroyed during the analytical process and so curatorial concerns may prohibit such analyses. A method in which calculus can be analysed for stable carbon and nitrogen concentrations has been described by Scott and Poulson.<sup>66</sup> The advantage of using calculus is that it is not an inherent part of the skeleton but a secondary material and thus may overcome curatorial concerns regarding preservation of the specimen.<sup>67</sup>

However, more recent research indicates that as the formation processes and composition of dental calculus can be highly variable among and within individuals, results from carbon and nitrogen isotopic analysis compared to those from bone can be inconsistent. Consequently, caution has to be exercised in using these results in dietary interpretations and subsequent conclusions may be invalid.<sup>68,69</sup>

## Trade and craft activities

During the process of biomineral maturation of dental plaque, not only are dietary microfossils entrapped within the calcifying matrix, but also a wide variety of airborne and waterborne debris. Among them can be waste products associated with craft and trade activities, which includes materials such as ground stone grit and plant and animal fibres.<sup>41</sup>

Lapis lazuli crystals have been found embedded within the calculus of a 9–14th century AD woman buried in a church-monastery complex at Dalheim, Germany. The blue particles were dispersed across many dental calculus fragments from different teeth, suggesting the particles entered the calculus in separate episodes rather than as a single localised event. Lapis lazuli is mined from a single region in Afghanistan and was a long-distance luxury trade good in the premodern era. Members of religious orders were the prime producers of books in the Middle Ages and lapis lazuli was used by the scribes and painters to illuminate high-quality texts.<sup>39</sup> Although it is not certain how the mineral came to be embedded in the calculus, it is known that craftspeople occasionally licked their brushes to make a fine point when embellishing manuscripts.<sup>70</sup>

Elemental analysis of crystals found in the dental calculus of an individual from an Etruscan-Celtic necropolis at Monterenzio Vecchia in Northern Italy revealed a very high level of manganese. This element is frequently found in ancient ceramic technology, such as pottery and paintings, and in this instance, could reflect work exposure to the pollutant.<sup>15</sup>

Evidence of preserved cellulose fibres, consistent with the characteristics of cotton, were found embedded within the matrix of dental calculus from four Late Woodland individuals (6–12th century AD) from the Danbury site, Ohio. The particular cotton fibres, *Gossypium* spp., are not indigenous to that region and would have to have been introduced from a south-western source. Such an interaction between Northern Ohio and southern coastal regions is supported by archaeological evidence of both cotton and marine shell exchange. How the cotton fibres became entrapped in the calculus is unclear but it is possible that the teeth were used as tools to separate the fibres when engaged in spinning cotton. Archaeological information of this nature is able to help shed new light on the craft industry and long-distance trade in prehistoric North America.<sup>71</sup>

## Ancient diseases

Not only is ancient calculus a rich reservoir of oral microbiota, food particles and other debris, but as mentioned, acquired pathogens are also trapped within its matrix. From a study of these ancient pathogens, it is possible to identify the origins, causes and evolution of specific infectious diseases. These opportunistic pathogens include those that are involved in periodontal, respiratory, cardiovascular and various other systemic diseases.<sup>28</sup>

One such condition is leprosy and evidence of the *Mycobacterium leprae* genome, the causative agent of leprosy, has been recovered via shotgun sequencing from the calculus of a sixteenth-century individual from Trondheim, Norway. The presence of *M. leprae* DNA and peptides in the calculus suggest an oral manifestation of the disease, considered perhaps to be the mucosa or soft palate. Calculus represents an alternative sample source to bones and teeth, especially in the absence of definite osteological markers and where human remains are poorly preserved or too valuable to warrant destructive bone sampling.<sup>72</sup>

Next-generation sequencing of calculus samples from an older woman at a prehistoric site in San Francisco Bay (CA-SCL-919) has revealed high levels of *Neisseria meningitidis*, one of the most common causes of bacterial meningitis. This, combined with the presence of incipient endocranial lesions and pronounced meningeal grooves, suggests an ancient case of meningococcal disease.<sup>73</sup>

Investigations into the calculus of a Roman woman, housed at the National Archaeological Museum of Cosa, Tuscany, has revealed what is considered to be the first historical evidence of coeliac disease. Molecular analyses demonstrated the HLA-DQ 2.5 haplotype, typically associated with a high predisposition to coeliac disease, while the results of the stable isotope analysis were suggestive of chronic malnutrition. Optical microscopic analysis revealed a gluten-rich diet and the skeletal remains displayed enamel hypoplasia and cribra orbitalia. In addition, there were specific molecular markers supporting the use of several medicinal herbal products, possibly aimed at treating this condition. One of these was for metabolites typical of exotic rhizomes, which are recognised for their anti-inflammatory and immunomodulatory properties. These were not native to Italy, instead perhaps coming from Eastern Asia, which would sustain historical information about the existence of trade routes at that time.<sup>74</sup>

## Conclusions

The build-up of dental calculus on teeth is an important oral health issue. In forensic studies, calculus has the potential to serve as a useful investigative resource. But, it is in the fields of anthropology and particularly archaeology that the analysis of ancient calculus has been revealed as having significant applications. Techniques have progressed considerably since its ability to inform on past human lives was first recognised. The quality and value of information that can be obtained from the identification of embedded microfossil remains and other debris is improving and expanding all the time.

Dental calculus is among the richest biomolecular source in the archaeological record and in recent years, biomolecular investigation of dental calculus has increasingly been utilised as an important tool in archaeological investigations. It is a hardy, long-term, biomolecular reservoir of ancient disease and dietary information and has important applications in the fields of medicine, archaeology and human evolutionary studies. Dental calculus is providing significant new insights and answers to questions, relevant to both archaeology and anthropology, and further advancements in this field can be expected.

### Ethics declaration

The author declares no conflicts of interest.

## References

- White D J. Dental calculus: recent insights into occurrence, formation, prevention, removal and oral health effects of supragingival and subgingival deposits. *Eur J Oral Sci* 1997; **105**: 508–522.
- Mann A E, Sabin S, Ziesemer K et al. Differential preservation of endogenous human and microbial DNA in dental calculus and dentin. *Sci Rep* 2018; **8**: 9822.
- Jepsen S, Deschner J, Braun A, Schwarz F, Eberhard J. Calculus removal and the prevention of its formation. *Periodontol* 2000 2011; **55**: 167–188.
- Rohanizadeh R, Legeros R Z. Ultrastructural study of calculus-enamel and calculus-root interfaces. *Arch Oral Biol* 2005; **50**: 89–96.
- Akali A, Lang N P. Dental calculus: the calcified biofilm and its role in disease development. *Periodontol* 2000 2018; **76**: 109–115.
- Dumitrescu A L, Kawamura M. Etiology of periodontal disease: Dental plaque and calculus. In Dumitrescu A L (ed) *Etiology and Pathogenesis of Periodontal Disease*. pp 1–38. Berlin: Springer, 2010.
- Balaji V R, Niazi T M, Dhanasekaran M. An unusual presentation of dental calculus. *J Indian Soc Periodontol* 2019; **23**: 484–486.
- Hinrichs J E, Thumbigere-Math V. The role of dental calculus and other local predisposing factors. In Newman M G, Takei H G, Klokkevold P R, Carranza F A (eds) *Newman and Carranza's Clinical Periodontology*. 13th ed. pp 190–207. Philadelphia: Elsevier, 2019.
- Mealey B L, Klokkevold P R. Impact of periodontal infection on systemic health. In Newman M G, Takei H G, Klokkevold P R, Carranza F A (eds) *Newman and Carranza's Clinical Periodontology*. 13th ed. pp 225–236. Philadelphia: Elsevier, 2019.
- Söder B, Meurman J H, Söder P-Ö. Dental calculus is associated with death from heart infarction. *Biomed Res Int* 2014; **2014**: 569675.



11. Söder B, Yakob M, Meurman J H, Andersson L C, Söder P-Ö. The association of dental plaque with cancer mortality in Sweden. A longitudinal study. *BMJ Open* 2012; DOI: 10.1136/bmjopen-2012-001083.
12. Preshaw P M, Alba A L, Herrera D *et al.* Periodontitis and diabetes: a two-way relationship. *Diabetologia* 2012; **55**: 21–31.
13. Higgins D, Austin J J. Teeth as a source of DNA for forensic identification of human remains: a review. *Sci Justice* 2013; **53**: 433–441.
14. Singh U, Goel S. Estimation and quantification of human DNA in dental calculus: A pilot study. *J Forensic Dent Sci* 2017; **9**: 149–152.
15. Charlier P, Huynh-Charlier I, Munoz O, Billard M, Brun L, de la Grandmaison G L. The microscopic (optical and SEM) examination of dental calculus deposits (DCD). Potential interest in forensic anthropology of a bio-archaeological method. *Leg Med (Tokyo)* 2010; **12**: 163–171.
16. Sørensen L K, Hasselström J B, Larsen L S, Bindsvlev D A. Entrapment of drugs in dental calculus – Detection validation based on test results from post-mortem investigations. *Forensic Sci Int* 2021; **319**: 110647.
17. Hardy K, Buckley S, Collins M *et al.* Neanderthal medics? Evidence for food, cooking, and medicinal plants entrapped in dental calculus. *Naturwissenschaften* 2012; **99**: 617–626.
18. Fiorin E, Sáez L, Malgosa A. Ferns as healing plants in medieval Mallorca, Spain? Evidence from human dental calculus. *Int J Osteoarchaeol* 2018; **29**: 82–90.
19. Gismondi A, D'Agostino A, Canuti L *et al.* Dental calculus reveals diet habits and medicinal plant use in the Early Medieval Italian population of Colonna. *J Archaeol Sci Rep* 2018; **20**: 556–564.
20. Allende M K-G, Samplonius A. Dental anthropological report: Exploring plant-based treatments through the analysis of dental calculus and sediment of dental caries in a woman from the Late Pre-ceramic period, Peru. *Ann Anat* 2022; **240**: 151849.
21. McDougall W A. Analytical transmission electron microscopy of the distribution of elements in human supragingival dental calculus. *Arch Oral Biol* 1985; **30**: 603–608.
22. Zhang B, Tan X, He X, Yang H, Wang Y, Zhang K. Evaluation of cadmium levels in dental calculus of male oral SCC patients with Betel-Quid chewing in Hunan Province of China. *Biol Trace Elem Res* 2019; **191**: 348–353.
23. Yaprak E, Yolcubal I, Sinanoğlu A, Doğrul-Demiray A, Guzeldemir-Akçakanat E, Marakoğlu I. High levels of heavy metal accumulation in dental calculus of smokers: a pilot inductively coupled plasma mass spectrometry study. *J Periodontol Res* 2017; **52**: 83–88.
24. Charlier P. Les dents d'Agnès Sorel. *L'Information dentaire* 2005; **25**: 1512–1513.
25. Lee R A, Herigon J C, Benedetti A, Pollock N R, Denkinger C M. Performance of saliva, oropharyngeal swabs, and nasal swabs for SARS-CoV-2 molecular detection: A systematic review and meta-analysis. *J Clin Microbiol* 2021; DOI: 10.1128/JCM.02881-20.
26. Berton F, Rupel K, Florian F, Biasotto M, Pallavicini A, Di Lenarda R. Dental calculus – a reservoir for detection of past SARS-CoV-2 infection. *Clin Oral Investig* 2021; **25**: 5113–5114.
27. Fuchs T A, Abed U, Goosmann C *et al.* Novel cell death programme leads to neutrophil extracellular traps. *J Cell Biol* 2007; **176**: 231–241.
28. Warinner C, Rodrigues J F, Vyas R *et al.* Pathogens and host immunity in the ancient human oral cavity. *Nat Genet* 2014; **46**: 336–344.
29. Ozga A T, Nieves-Colón M A, Honap T P *et al.* Successful enrichment and recovery of whole mitochondrial genomes from ancient human dental calculus. *Am J Phys Anthropol* 2016; **160**: 220–228.
30. Henry A G, Piperno D R. Using plant microfossils from dental calculus to recover human diet: a case study from Tell al-Raqqā'i, Syria. *J Archaeol Sci* 2008; **35**: 1943–1950.
31. Power R C, Salazar-García D C, Wittig R M, Henry A G. Assessing use and suitability of scanning electron microscopy in the analysis of micro remains in dental calculus. *J Archaeol Sci* 2014; **49**: 160–169.
32. Hardy K, Radini A, Buckley S *et al.* Dental calculus reveals potential respiratory irritants and ingestion of essential plant-based nutrients at Lower Palaeolithic Qesem Cave Israel. *Quatern Int* 2016; **398**: 126–135.
33. D'Agostino A, Di Marco G, Rubini M *et al.* Environmental implications and evidence of natural products from dental calculi of a Neolithic-Chalcolithic community (central Italy). *Sci Rep* 2021; **11**: 10665.
34. Dobney K, Brothwell D. Dental calculus: its relevance to ancient diet and oral ecology. In Cruwys G, Foley R (eds) *Teeth and Anthropology*. pp 55–81. Oxford: British Archaeological Reports International Series, 1986.
35. Dobney K, Brothwell D. A scanning electron microscope study of archaeological dental calculus. In Olsen S L (ed) *Scanning Electron Microscopy in Archaeology*. pp 372–385. Oxford: British Archaeological Reports International Series, 1988.
36. Lieverse A R. Diet and the aetiology of dental calculus. *Int J Osteoarchaeol* 1999; **9**: 219–232.
37. Wesolowski V, de Souza S M F M, Reinhard K J, Ceccantini G. Evaluating microfossil content of dental calculus from Brazilian sambaquis. *J Archaeol Sci* 2010; **37**: 1326–1338.
38. Henry A G, Brooks A S, Piperno D R. Microfossils in calculus demonstrate consumption of plants and cooked foods in Neanderthal diets (Shanidar III, Iraq; Spy I and II, Belgium). *Proc Natl Acad Sci U S A* 2011; **108**: 486–491.
39. Radini A, Tromp M, Beach A *et al.* Medieval women's early involvement in manuscript production suggested by lapis lazuli identification in dental calculus. *Sci Adv* 2019; DOI: 10.1126/sciadv.aau7126.
40. De la Fuente C P, Flores S V, Moraga M L. Human bacterial DNA from dental calculus: a new source of genetic material. *Am J Phys Anthropol* 2012; **147**: 127.
41. Warinner C, Speller C, Collins M J. A new era in palaeomicrobiology: prospects for ancient dental calculus as a long-term record of the human oral microbiome. *Philos Trans R Soc Lond B Biol Sci* 2015; **370**: 20130376.
42. Mackie M, Radini A, Speller C. The sustainability of dental calculus for archaeological research. In Favreau J, Patalano R (eds) *Shallow Pasts, Endless Horizons: Sustainability & Archaeology: Proceedings of the 48th Annual Chacmool Archaeology Conference*. pp 74–81. Calgary: The Chacmool Archaeological Association of the University of Calgary, 2017.
43. Fagnäs Z, García Collado M I, Hendy J *et al.* A unified protocol for simultaneous extraction of DNA and proteins from archaeological dental calculus. *J Archaeological Sci* 2020; **118**: 105135.
44. Hardy K, Blakeney T, Copeland L, Kirkham J, Wrangham R, Collins M. Starch granules, dental calculus and new perspectives on ancient diet. *J Archaeol Sci* 2009; **36**: 248–255.
45. Weyrich L S, Dobney K, Cooper A. Ancient DNA analysis of dental calculus. *J Hum Evol* 2015; **79**: 119–124.
46. Lippi M M, Pisaneschi L, Sarti L, Lari M, Moggi-Cecchi J. Insights into the Copper-Bronze Age diet in Central Italy: Plant microremains in dental calculus from Grotta dello Scoglietto (Southern Tuscany, Italy). *J Archaeol Sci Rep* 2017; **15**: 30–39.
47. D'Agostino A, Canini A, Di Marco G, Nigro L, Spagnoli F, Gismondi A. Investigating plant micro-remains embedded in dental calculus of the Phoenician inhabitants of Motya (Sicily, Italy). *Plants (Basel)* 2020; **9**: 1395.
48. Jovanović J, Power R C, de Becdelièvre C, Goude G, Stefanović S. Microbotanical evidence for the spread of cereal use during the Mesolithic-Neolithic transition in the Southeastern Europe (Danube Gorges): Data from dental calculus analysis. *J Archaeol Sci* 2021; **125**: 105288.
49. Kinaston R, Willis A, Miskiewicz J J, Tromp M, Oxenham M F. The dentition: Development, disturbances, disease, diet and chemistry. In Buikstra J E (ed) *Ortner's Identification of Pathological Conditions in Human Skeletal Remains*. 3rd ed. pp 749–797. London: Academic Press, 2019.
50. Curvers H H, Schwartz G M. Excavations at Tell al-Raqqā'i: a small rural site of early urban northern Mesopotamia. *Am J Archaeol* 1990; **94**: 3–23.
51. Cristiani E, Radini A, Borčić D *et al.* Dental calculus and isotopes provide direct evidence of fish and plant consumption in Mesolithic Mediterranean. *Sci Rep* 2018; **8**: 8147.
52. Power R C, Salazar-García D C, Straus L G, González Morales M R, Henry A G. Microremains from El Mirón Cave human dental calculus suggest a mixed plant-animal subsistence economy during the Magdalenian in Northern Iberia. *J Archaeol Sci* 2015; **60**: 39–46.
53. Lazaridis I, Patterson N, Mittnik A *et al.* Ancient human genomes suggest three ancestral populations for present-day Europeans. *Nature* 2014; **513**: 409–413.
54. Allentoft M E, Sikora M, Sjögren K-G *et al.* Population genomics of Bronze Age Eurasia. *Nature* 2015; **522**: 167–172.
55. Haak W, Lazaridis I, Patterson N *et al.* Massive migration from the steppe was a source for Indo-European languages in Europe. *Nature* 2015; **522**: 207–211.
56. Dominguez-Bello M G, Blaser M J. The human microbiota as a marker for migrations of individuals and populations. *Annu Rev Anthropol* 2011; **40**: 451–474.
57. Adler C J, Dobney K, Weyrich L S *et al.* Sequencing ancient calcified dental plaque shows changes in oral microbiota with dietary shifts of the Neolithic and Industrial revolutions. *Nat Genet* 2013; **45**: 450–455.
58. Eisenhofer R, Anderson A, Dobney K, Cooper A, Weyrich L S. Ancient microbial DNA in dental calculus: A new method for studying rapid human migration events. *J Isl Coast Archaeol* 2019; **14**: 149–162.
59. Aufderheide A C, Rodriguez-Martin C, Langsjoen O. *The Cambridge Encyclopedia of Human Paleopathology*. Cambridge: Cambridge University Press, 1988.
60. Yu H-H, Lee D-H, Seo S-J, You Y-O. Anticariogenic properties of the extract of *Cyperus rotundus*. *Am J Chin Med* 2007; **35**: 497–505.
61. Najah A M. *In vitro* Inhibitory effect of *Cyperus rotundus* L crude extracts on mouth isolates of *Streptococcus mutans* and *Candida albicans*. *Al-Mustansiriyah J Pharma Sci* 2012; **11**: 85–91.
62. Buckley S, Usai D, Jakob T, Radini A, Hardy K. Dental calculus reveals unique insights into food items, cooking and plant processing in Prehistoric Central Sudan. *PLoS One* 2014; DOI: 10.1371/journal.pone.0100808.
63. Cummings L S, Yost C, Soltysiak A. Plant microfossils in human dental calculus from Nemrik 9, a Pre-Pottery Neolithic site in Northern Iraq. *Archaeol Anthropol Sci* 2018; **10**: 883–891.
64. Schoeninger M J. Stable isotope evidence for the adoption of maize agriculture. *Curr Anthropol* 2009; **50**: 633–640.
65. Schoeninger M J. Diet reconstruction and ecology using stable isotope ratios. In Larsen C S (ed) *A Companion to Biological Anthropology*. pp 445–464. Chichester: Wiley-Blackwell, 2010.
66. Scott G R, Poulson S R. Stable carbon and nitrogen isotopes of human dental calculus: a potentially new non-destructive proxy for paleodietary analysis. *J Archaeol Sci* 2012; **39**: 1388–1393.
67. Forshaw R J. Dental indicators of ancient dietary patterns: dental analysis in archaeology. *Br Dent J* 2014; **216**: 529–535.
68. Salazar-García D C, Richards M P, Nehlich O, Henry A G. Dental calculus is not equivalent to bone collagen for isotope analysis: a comparison between carbon and nitrogen stable isotope analysis of bulk dental calculus, bone and dentine collagen from same individuals from the Medieval site of El Raval (Alicante, Spain). *J Archaeol Sci* 2014; **47**: 70–77.
69. Price S D R, Keenleyside A, Schwarcz H P. Testing the validity of stable isotope analyses of dental calculus as a proxy in paleodietary studies. *J Archaeol Sci* 2018; **91**: 92–103.
70. Coccato A, Moens L, Vandenabeele P. On the stability of medieval inorganic pigments: A literature review of the effect of climate, material selection, biological activity, analysis and conservation treatments. *Herit Sci* 2017; **5**: 12.
71. Blatt S H, Redmond B G, Cassman V, Scullip P W. Dirty teeth and ancient trade: Evidence of cotton fibres in human dental calculus from Late Woodland, Ohio. *Int J Osteoarchaeol* 2011; **21**: 669–678.
72. Fotakis A K, Denham S D, Mackie M *et al.* Multi-omic detection of *Mycobacterium leprae* in archaeological human dental calculus. *Philos Trans R Soc Lond B Biol Sci* 2020; **375**: 20190584.
73. Eerkens J W, Nichols R W, Murray G G R *et al.* A probable prehistoric case of meningococcal disease from San Francisco Bay: Next generation sequencing of *Neisseria meningitidis* from dental calculus and osteological evidence. *Int J Paleopathol* 2018; **22**: 173–180.
74. Gismondi A, D'Agostino A, Di Marco G *et al.* Back to the roots: dental calculus analysis of the first documented case of coeliac disease. *Archaeol Anthropol Sci* 2020; **12**: 6.