

Forensic Anthropological Significance of Dental Calculus Deposits as Proxy Identifier of the Host and the Oral Microbiota: A Scoping Review

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ABSTRACT: Dental calculus is a creamish-yellow to brownish-black hard crust deposited on teeth, having the dietary micro-remains, biomolecules, oral microbes and the secretions preserved in it over a long period of time. It has served as a valuable source material for research in diverse scientific disciplines such as anthropology, archaeology, microbiology and forensic sciences. The host and microbial DNA extracted and sequenced from dental calculus deposits (DCD) have helped to establish the identity of unknown individuals, and also the use of certain drugs, tobacco products by the individuals of the past as well as contemporary human populations. The entrapped cellular as well as tissue fragments in calculus can help in identification, reconstruction of dietary habits, food practices, manner of death/pathologies the geographical and occupational affinity of ancient human remains. Calculus deposits gleaned from the archaeological or paleontological specimens can be used to assess the shifts in oral microbiota compositions and host-pathogen co-evolution as researchers have found calculus as rich source of oral microbiomes, pathogens, dietary biomolecules, and host DNA. Advancing dental calculus research through validation studies, technological innovations, interdisciplinary collaborations, longitudinal research, and ethical considerations holds promise for its robust forensic anthropological utilizations. The current status of anthropological, archaeological and microbial research involving dental calculus deposits, future challenges, and its forensic anthropological significance are presented in this review article.

KEYWORDS: forensic anthropology, dental calculus, dietary and disease status, host and oral microbiome DNA, identification



Original article

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Introduction

Reconstructing life-histories and health status of past human populations has remained a major subject of discussions and debates in the anthropological research domain (Wasterlain et al. 2011). Teeth and bones have served as excellent reservoirs of biomaterials, appropriately suitable for identification of unknown human remains and/or missing persons. Among osseous human remains, teeth are the strongest structures generally found well preserved in most forensic anthropological contexts (Raj et al. 2013). During the routine lifetime of an individual, the oral microbiome and the micro-debris circulating in human oral cavity often build up to preserve as a tightly adhered calcified dental plaque, called dental calculus deposits (DCD). Dental calculus is a creamish-yellow to brownish-black hard crust deposited on the inner surface of tooth or the dental prosthesis. The calcified diverse bacterial biofilm (i.e., DCD) structurally preserves and protects bacterial cells from multiple external factors more precisely than any other substrate, and thus has potential to reveal the living conditions of an individual (Willmann et al. 2018). Dental calculus is a densely mineralized microbial biofilm formed from assimilation of mineral salts in salivary and gingival fluids and it harbors the dietary, microbial and environmental information about an individual (Hardy et al. 2009; Buckley et al. 2014; Warinner and Lewis 2015; Weyrich et al. 2015; Gismondi et al. 2018). The nature of material and microbial remnants entrapped in calculus depends upon the lifestyle, oral hygiene, dietary habits, environmental conditions, genetics, disease and health status such as periodontitis and dental crowding of the host (Weyrich et al. 2015;

Singh and Goel 2017; Fons-Badals et al. 2020; Blatt et al. 2022). Dietary, microbial and host relationships can be conveniently established by examining only one source material i.e., dental calculus (Weyrich et al. 2015; Mann et al. 2018; Ottoni et al. 2019; Ozga et al. 2019; Modi et al. 2020).

Calculus is a sturdy and rigid material that can be collected non-invasively from jaw fragments and/or tooth surfaces. Even very minute quantities of calculus can provide deep insights about the geolocality, ecology and socio-cultural affinity of an individual; thus, presenting a more holistic ante-mortem profile of the unidentified individuals (Mackie et al. 2017; Blatt et al. 2022). Dental calculus serves as a vital bio-material in situations where only heavily decomposed or badly degraded, damaged and fragmented human remains of disturbed skeletal conformity are available for forensic anthropological identifications. Thus, it can be taken as a compelling biological material having crucial forensic implications, especially in identification of heavily damaged and severely degraded human remains.

Medically, the development and accumulation of dental calculus is considered an oral health problem by periodontists, so calculus deposits are routinely removed to maintain sound dental health, and are discarded as a clinical waste material. However, its scientific scrutinization has significant forensic anthropological and bio-archaeological implications. Its deposition below the cervical line may be the indicative of periodontitis or other non-infectious diseases which, in turn, may have different forensic or bio-archaeological interpretations (Muro and Cucina 2024). Building up of calculus deposits usually compromises oral hygiene

by promoting increments in pathogenic plaque, resulting into excessive destruction of periodontal tissues, thus enhanc-

ing the risks of systemic diseases like diabetes (Preshaw et al. 2012; Mealey and Klokkevold 2019).



Figure 1. Anthropological importance of dental calculus

In addition to mineral components, calculus contains a variety of inorganic and organic remnants (of salivary, dietary or bacterial origin) incorporated either during the mineralization or post-calcification of the calculus. It has been reported to be present on the supra- and/or sub-lingual surfaces of teeth. Supra-gingival calculus is primarily anchored to the mandibular lingual surfaces of anterior teeth and the buccal surfaces of maxillary molars, whereas the sub-gingival calculus is found adhered to the entire set of teeth, specifically on their proximal tooth surfaces. Formation and accumulation of dental calculus also varies with tooth type; maxillary molars and mandibular incisors being more prone to supra-lingual calculus (White 1997). Further, removal of calculus from teeth

of skeletal remains is non-invasive and comparatively less destructive than the traditional method of DNA extraction and analysis in such contexts.

The prevalence of calculus deposits has been reported among individuals of almost all known human populations (ancient or contemporary); though its formation and presence is highly population-specific (Warriner et al. 2015; Mann et al. 2018). The frequency distribution of calculus deposits is largely dependent upon the available living conditions of the affected individual, such as oral hygiene (Anerud et al. 1991; Blank et al. 1994; MacPherson et al. 1995), age (Beiswanger et al. 1989; Anerud et al. 1991; MacPherson et al. 1995), sex (Beiswanger et al. 1989; Anerud et al. 1991), ethnicity, diet (Schroeder 1969; Bhat 1991), dental

cares (Beiswanger et al. 198; Anerud et al. 1991; Blank et al. 1994; MacPherson et al. 1995), systemic disease (Emrich et al. 1991), medicine intake (Turesky et al. 1992; Breuer et al. 1996), educational status, as some examples. In non-westernized populations, calculus formation starts soon after tooth eruption and is found to continue up to the maximum age of 30 years (White 1997), however, no such trends have been reported for the individuals of westernized world.

Dental calculus undergoes continuous, though periodic mineralization to incorporate oral microbiota, phytoliths, pollens, fat grains and toxic micro-remains into it (Putrino et al. 2024), and acts as an authentic and prolonged storehouse of such remnants (Jin and Yip 2002; Adler et al. 2013; Warinner et al. 2013). Food and non-food micro-particles like starch granules, pathogens, parasites, pollens, diatoms, seeds, hair, dietary/vegetal fibers, cereals, the accidentally entrapped small insects, mineral salts and crystals are frequent inclusions in human dental calculus matrix (Dobney and Brothwell 1986; 1988). The plant remnants in the form of phytoliths and starch grains have potential utility in estimating ancient dietary habits as well as population-level food variations. Starchy foods constitute 50–70% of energy intake in modern as well as pre-agriculturist human diets (Hardy et al. 2009). The sophisticated dietary regime of Neanderthals, primarily based on plant-based foods, has been revealed through dental calculus (Henry et al. 2011). Dental calculus extracted from ancient and modern dental remains has been widely used for studying the composition and profiling of oral microbiota, remnant food debris, disease microbes entrapped within calcium phosphate mineral salts of saliva and

dental plaque (Henry and Piperno, 2008; Henry et al. 2011; Warinner et al. 2014).

The aim of this article is to provide an updated review focused on the formation, prevalence, composition and forensic anthropological significance of dental calculus deposits.

Methods

To scrutinize the current status of dental calculus research for forensic anthropological purposes, scientific databases were searched using search engines including: PubMed, ScienceDirect, SAGE, Springerlink, Clinical Key, WoS and Google-Scholar, using the following key terms: 'Dental calculus, Forensic identification', 'Microbial forensics and oral microbiome', 'Ancient DNA and dental calculus', 'Occupation and dietary status from calculus, Oral microbiota and forensic odontology, 'Dental calculus and stable isotope analysis'. Snowball sampling technique of cross-referencing was used to identify related articles and the PRISMA guidelines were followed to include eligible research articles. Full-text articles published until 2022, and containing information relevant to the scope of present systematic review were further analyzed in-depth. The inclusion criteria considered studies related to forensic identification based on dental calculus and those published until 2022. Studies not satisfying the inclusion criteria were excluded from further analyses.

A total of 89 research articles were identified through search from the above scientific databases. Thirty studies were removed from consideration of further analysis as they were either modified or unrelated to the scope of present review, published in languages other than English, or marked as 'ineligible' by the an-

notation tools. Out of 59 articles selected on the basis of their title and abstract, another 16 studies were neglected as being duplicates, reviews, or not commenting upon the accuracy of dental calculus for forensic anthropological purposes. Another

set of 16 studies were discarded for final analyses as their full-text version could not be found or arranged for scrutinization and analysis. Finally, 31 studies were found within the ambit/scope of the aim and objectives of present study (Figure 2).

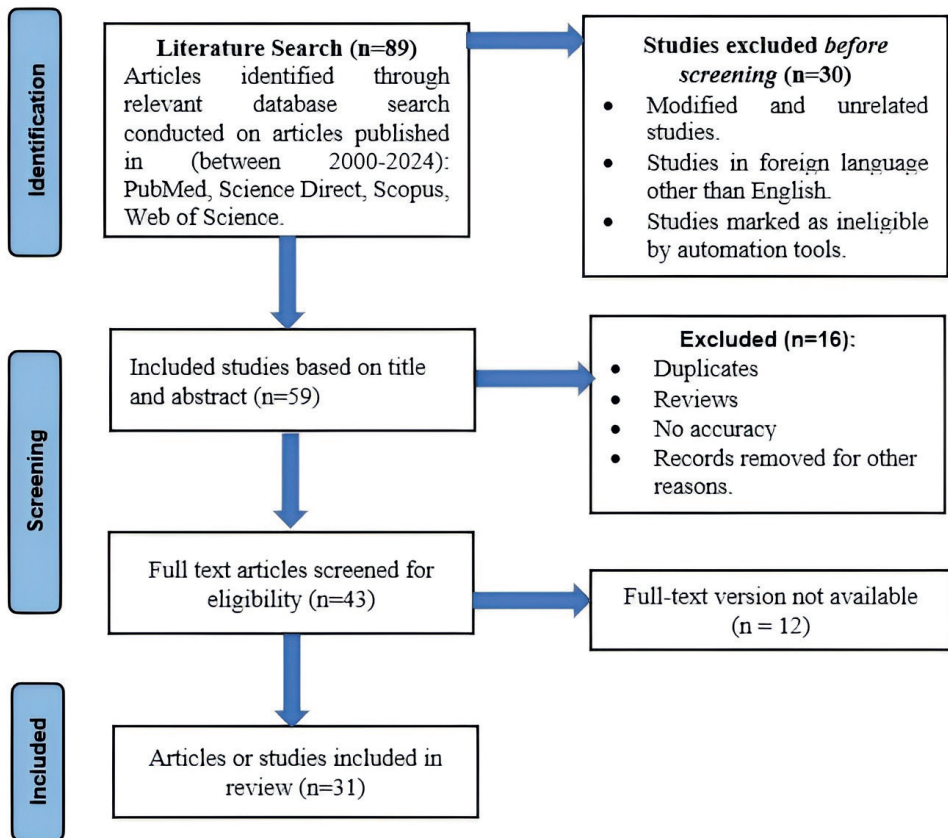


Figure 2. Flowchart showing stages of selection of articles for systematic review

Results

The systematic review revealed that dental calculus is a promising biological material for establishing identity, assessing health and dietary status, estimating geo-affinity, exposure to heavy metals or drugs of

abuse and establishing other identity credentials of an unknown individual. It serves as a rich source of host-associated biomolecules, ingested or inhaled during the routine daily life of an individual. The present systematic review found that the majority of dental calculus studies were

reported in Europe where the dental remains from archaeological sites and cemeteries were most commonly used for the purpose. Only one pilot study (Singh and Goel 2017) was reported from India. The literature review indicated that the majority of calculus research is limited to reporting its quantity and location on teeth, the microbiota preserved in it, its clinical and pathological significance and forensic importance for identifying archaeological or contemporary human remains (Brothwell 1981; Dobney and Brothwell, 1988). The type of teeth or the site (supra-/sub-lingual) from where calculus was collected for analysis was not mentioned in most of the studies. Until very recently, the scientific importance of dental calculus for forensic anthropological casework was not recognized as it could not attract the desired attention of the scientific community. Calculus has been appreciated as a significant population and individuality marker as it abundantly preserves the host-associated routine micro-particles and biomolecules in it (Radini et al. 2017). The observations of present systematic review can be summarized in the following categories.

Collection and analysis of dental calculus

The proper collection, sampling, preparation and analysis of dental calculus is generally done according to the standardized established protocols. Dental calculus can be collected from ancient as well as modern teeth/jaw fragments in individuals reporting to dental clinics for their routine dental check-up such as cleansing or screening purposes. Enough dental calculus can be easily collected either from the lingual or buccal surfaces of different mandibular as well as maxillary teeth without scratching or scraping tooth surfaces (Scott and Poulson, 2012). Two 3-5 mm long fragments of supra-lingual calculus

are generally removed (particularly from 2 to 3 maxillary molars or mandibular incisors) using sterile or decontaminated hand or ultrasonic scaler (White 1997; Velsko et al. 2019). For forensic identification purposes, the supragingival calculus is preferred to the sublingual one as the latter is heavily mineralized, contaminated with gingival haemorrhagic components and is tightly anchored to the teeth. Detailed demographic, nutritional and disease information of an individual is crucial for objective analysis of calculus samples for anthropological or forensic purposes.

The material, molecular and chemical information entrapped in calculus deposits can be analyzed with multiple morphoscopical, analytical, cytological, histological, biochemical, molecular and optical techniques. The more sophisticated methods include optical microscopy, scanning electron microscopy, energy-dispersive x-ray spectroscopy, ultra-performance liquid chromatography-mass spectrometry, to provide information about not only identity credentials of an individual (Dobney and Brothwell 1986; 1988; Lieverse 1999; Henry et al. 2011; De la Fuente et al. 2012; Radini et al. 2017; Strömberg et al. 2018; Fotakis et al. 2020; Godoy Allende and Samplonius 2022) but also for quantifying traces of nicotine alkaloids in calculus for better understanding the consumption of such intoxicants by the smokers (Eerkens et al. 2018). The entrapment and preservation of the cellular fragments, starch granules and other inclusions in dental calculus can be examined by microscopic and spectroscopic techniques (Henry and Piperno 2008; Charlier et al. 2010; Power et al. 2015). Dosseto et al. (2024) reported that measuring strontium (Sr) isotopes from fossil calculus using multicollector inductively coupled plasma mass spectrometry helped

in correctly identifying the composition of soil where the remains were buried which, in turn, helped in estimating the geolocality of unknown human remains.

Forensic archaeological reconstructions

Microscopic examination of calculus deposits is highly useful in providing decisive data for forensic or medico-legal provenance or identity establishment purposes (Figure 3). Dental calculus deposition is generally considered as an ectopic growth which provides an alternate to the destructive processing of human skeletal remains. Cultural identity, individualized habits (smoking/drugs/tooth-brushing), occupational hazards (painting, mining, farming), disease or pollution exposures, even COVID-19 infections of an individual can be objectively assessed from the trace elemental analysis of dental calculus (Bergstrom 1999; Radini et al. 2017; Yaprak et al. 2017; Eerkens et al. 2018; Sørensen et al. 2021; Berton et al. 2021; Li et al. 2022). Phytoliths and plant fibers entrapped in dental calculus can help in the identification of eaten plant sources, the season or

environment in which they grew, the travel routes followed and the burial location of unknown individuals (Blatt et al. 2011); thus, it can also serve as possible geographical and occupational marker of identity of an individual. The particles embedded in calculus can help in identifying occupations such as metal-workers, carpenters, and other artisans. Heavy metals deposited in calculus of production workers can be used as occupational exposure marker (Abdazimov 1991). Researchers have found that drug residues are better entrapped in the interior of the calculus material than in blood, and few drugs are found in comparatively higher concentrations in dental calculus (Sørensen et al. 2021). Thus, the manner of death, work-related intoxication or individualized habits can be reconstructed from the toxicological analysis of calculus (Charlier et al. 2010). Among the typical sources of endogenous DNA, saliva, oral fluids, proteins and mucosal or epithelial cells are found commonly found embedded in dental calculus, endorsing the possibilities of isolating mtDNA from dental calculus.

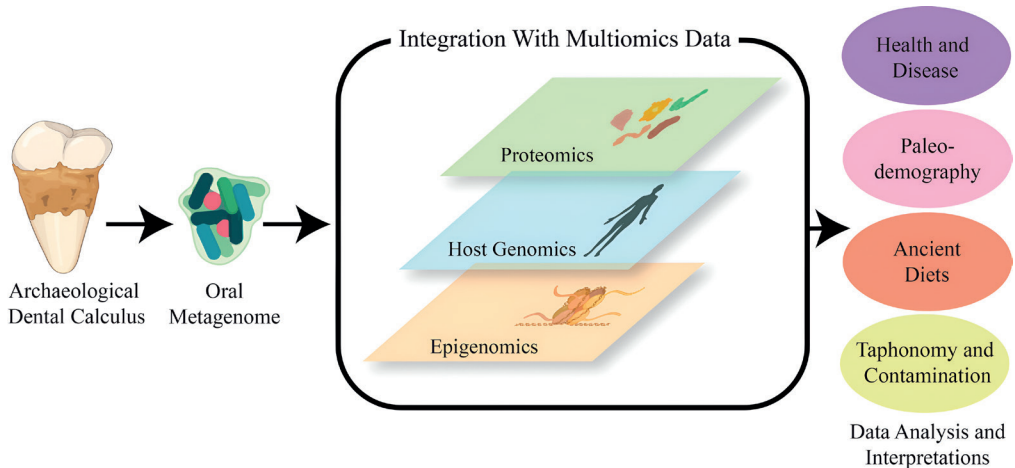


Figure 3. Multiple applications of archaeological dental calculus (reprint used with Open Access permission from Wright et al. 2021)

DNA from dental calculus: deciphering host-oral microbiota interactions

Calculus is a calcified form of oral microbial plaque biofilm and is a rich source of host and oral microbiome DNA (Preus et al. 2011; Brealey et al. 2020). It has been widely used as a tool in microbial forensics to estimate microbial health and the oral hygiene of the deceased. The comparative analysis of DCD from ancient and modern dentition can help understand the issues related to the abundance and diffusion of different microorganisms in the environments of the past or contemporary biospheres (Brundin et al. 2013; Weiß et al. 2020). The host mt-DNA and bacterial DNA, along with micro-particles entrapped in calculus matrix can be conveniently extracted, sequences and analyzed using advanced bio-molecular, spectroscopic or microscopic methods which, in turn, have revolutionized our understanding of the identity, diets, ancestry, occupations, migration patterns, disease, pollutant/metallic exposures, health and environmental conditions of past individuals and the populations. The molecular and chemical analysis of dental calculus can help understand the intricate dynamic relationship that existed between humans and their microbes (Warinner et al. 2015). Recent improvements in genome sequencing strategies, bioinformatics armamentarium, laboratory workflow designs and contamination controls have significantly enhanced the potential of ancient microbial research (Gilbert et al. 2005; MacLean et al. 2009; Kaczynski et al. 2012; Weyrich et al. 2015).

Microbial cells constitute about 70% of total dry weight of calculus, highlighting that the majority of calculus DNA is microbial in nature (Aas et al. 2005; Dewhirst et al. 2010; Human Microbi-

ome Project Consortium 2012; Warinner et al. 2014; Velsko et al. 2019; Kazarina et al. 2021; Ottoni et al. 2021). The constantly evolving microbes have helped in laboratory studies of bacterial evolution which, in turn, has increased our information about the dynamics of evolution, adaptive changes, and respond to queries impacting human health (Bonczarowska et al. 2022). Ancient oral microbiota is considerably more heterogeneous than the modern one and this heterogeneity of ancient oral microbiome composition can offer more insights into human evolution (Jersie-Christensen et al. 2018) as good oral hygiene discourages calculus formation (2022). The dental calculus harbors past human oral microbiota that can provide deep insights into decedents' lifestyle, diet, health, disease, possible geolocation, environmental conditions and the cultural affiliation of an individual; thus presenting a more comprehensive ante-mortem profile of the unknown ones (Blatt et al. 2022), even from its minute quantities (Mackie et al. 2017). The application of advanced genetic techniques to detect microbial communities in ancient skeletal remains has revolutionized paleopathological research towards pathogenic evolution and identification from profiling of human oral microbiome (Bos et al. 2019; Arning and Wilson 2020; Li et al. 2022; Naud et al. 2022).

Human oral microbiome research based on dental calculus and transitions in oral microbiota has significantly contributed to our understanding of the functional diversity of microbial ecologies, oral diseases, interactions between humans, microbes and the abiotic factors the during lifetime of an individual (Warinner et al. 2015). It is the mineralization process of calculus happening during life that makes it resistant to environmen-

tal contamination and lets the microbial community get preserved in it (Jin and Yip, 2002). The temporal and spatial variations in oral microbiota can provide crucial information not only to the forensic anthropologists and archaeologists, but also to medical researchers like microbiologists, and evolutionary biologists. Microbial evolution provided deep insights into major changes in human condition like diseases, epidemiological transitions, biogeographic range expansions, colonialism and industrialization (Warinner et al. 2015; Blatt et al. 2022).

Detection of drugs and related metabolites

Plant-based pharmaceutical or psychoactive substances and other stimulants have been commonly used in modern as well as ancient times, particularly for treatment of some medical conditions (Fiorin et al. 2018; Gismondi et al. 2018; Godoy Allende and Samplonius 2022). Revealing smoking or chewing habits, use of pharmaceutical products, illicit psychoactive drugs/stimulants and their metabolites by past populations is a crucial forensic archaeological inquiry. Recovery of plant food debris from archaeological dental calculus has helped in reconstruction of past food practices. Sørensen et al. (2021) reported that such plant inclusions found entrapped and preserved in dental calculus can be easily detected and quantified. Dental calculus can also be helpful for monitoring betel nut chewing or tobacco smoking (containing numerous heavy metal) exposure to the human oral cavity (Yaprak et al. 2017; Zhang et al. 2019). Many instances have been reported in literature when the craft and trade related by-products like stone crystals, cellulose fibers or ceramics have been found preserved in dental calculus

to provide information about such practices in ancient populations worldwide (Blatt et al. 2011; Coccato et al. 2017). Supragingival dental calculus is the best choice for monitoring heavy metal (like cadmium, mercury) exposures to oral cavity which helps in assessing the risks of oral cancers due to heavy metal poisoning (Zhang et al. 2019; Charlier 2013), specifically using the analytical techniques like transmission electron microscopy (McDougall 1985).

Though dietary reconstructions from calculus have been widely studied, relatively fewer studies have considered the deposition of pharmaceutical or phototherapeutic residues in DCD (Hardy et al. 2018; Gismondi et al. 2018). In post-mortem cases, calculus may serve as a valuable adjunct to the existing analyses where traditional investigations do not provide any leads. Direct oral ingestion of substances, inhalation of smoke/vapor, and/or the release of cellular or serum residuals into the saliva and gingival crevicular fluid may facilitate entrapment and preservation of a large variety of pharmaceutical and psychoactive drugs in dental calculus. The variations in entrapment and deposition of this matter in calculus from different individuals may depend upon differences in the diet, health, local pH, salivary flow, and, lastly, the stability of the substances entrapped in the calculus (Sorensen et al. 2021). Use of medicinal plants in ancient times has been deciphered from chemical indicators in archaeological dental calculus samples (Hardy et al. 2009; Gismondi et al. 2018). Smoking or chewing of tobacco (nicotine) or any other product having skewed concentrations of toxic heavy metals like arsenic, mercury, silicon, lead or cadmium have been revealed from archaeological calculus

(Yaprak et al. 2017; Eerkens et al. 2018; Zhang et al. 2019); inductively coupled plasma mass spectrometry analysis of dental calculus being a novel non-invasive technique for analysis of environmental exposure to heavy metals (Zhang et al. 2015, 2019). Drug residues of cocaine, heroin, 6-monoacetylmorphine (6-MAM) and tetra-hydrocannabinolic acid A (THCA-A) have been found trapped in the interior of the modern calculus material (Sorensen et al. 2021). Saliva has higher affinity to accumulate basic drugs in higher concentrations than the blood owing to generally lower pH of the former.

Discussion

Until recently, the anthropological, archaeological and forensic research potential of calculus was not recognized fully, and it was overlooked with calculus being a discarded material. It is now fully understood that dental calculus houses crucial information about the past human life in the form of host-associated micro-particles and biomolecules embedded within its hardened and rough matrix, ingested/inhaled (intentionally or accidentally) during routine daily life of an individual (Buckley et al. 2014; Radini et al. 2017). It is the porous nature of DCD which makes it ideal for a variety of research. It has been recently recognized as an informative material to understand ancient diet and health in archaeological sciences (Forshaw 2022). It has immensely helped in revealing human prehistory, dietary variations and shifts, and disease signatures from ancient oral microbiome analysis of past populations (Hansen et al. 1991; Dobney 1994). The material and molecular information contained within DCD is vast and ever-expanding and it

can prove more useful than the bones in the identification of fragmented or burnt human remains.

Anthropological and molecular examinations of dental calculus collected from historical, archaeological, or forensic specimens may help in biological profiling, establishing cultural and geographical identity of such unknown human remains as calculus contains DNA in sufficient quantity required for such identifications and revealing microbial and host-DNA diversity (Lisman et al. 2023). Dental calculus is a rich reservoir of ancient host biomolecules and human DNA; both mitochondrial and nuclear DNA (Warinner et al. 2015; Singh and Goel 2017; Mann et al. 2018; Lisman et al. 2023). It is the rough and porous surface of the calculus deposits which promotes bacterial deposition, sometimes under standard hygiene conditions. It is this unique structure and mineralized nature of calculus that strongly embodied DNA within it and prevents the acidic or exogenous microbial attacks in the hardened matrix of calculus (Warinner et al. 2014; Mann et al. 2018). Genomics and forensic experts are well acquainted with the advantages of analyzing dental calculus which can be conveniently obtained from the living and recently dead individuals or even the skeletonized archaeological human remains (Singh and Goel, 2017). Dental calculus may contain up to 1000 times more DNA than any other skeletal element and has huge potential to revolutionize ancient biomedical research. It can be used as an alternative source material for ancient human DNA analyses when other skeletal tissues do not yield the desired results (Warinner et al. 2014; Forshaw, 2022). Dental calculus serves as an adjuvant and non-invasive evidence in

forensic human identification by establishing human-host DNA profile, living conditions, behavior, lifestyle, and the dietary status of the decedent (Blatt et al. 2022; Bonczarowska et al. 2022).

Highly degraded and challenged samples generally contain low amounts of DNA (with PCR inhibitors), which presents a very tough task for their forensic identification pursuits. Calculus provides a clean, stable, durable and hospitable environment for microbial communities, food and non-food particles and thus, serves as a viable source of forensic identification based on autosomal and Y-STR markers extracted from it (Sawafuji et al. 2020, Lisman et al. 2023). Human DNA bound in calculus hydroxyapatite is comparatively well preserved; thus it can be taken as potential investigative tool for forensic purposes (Higgins and Austin, 2013). The gingival secretions, shed epithelial cells, macrophages and oral inflammatory processes are the probable sources of DNA incorporation into dental calculus deposits (Warinner et al. 2015; Mann et al. 2018). Calculus DNA is relatively low in quantity and is fragmented, with the highest concentration found in subgingival plaque. The majority of available dental calculus research is primarily focused on estimating dietary habits, oral microbiota and mtDNA haplogroup affiliations of archaeological human remains (Black et al. 2011; Damle 2016).

The quality of DNA isolated from calculus significantly differs from that obtained from teeth. Dentine DNA is more degraded and damaged than the fragmented and shorter length (but more stable and less challenged) DNA obtained from dental calculus. A comparison of DNA yields from paired dental calculus and dentine samples from the same tooth of an individual has endorsed

that dental calculus is the richest known source of ancient biomolecules in human hard tissues (Willmann et al. 2018; Li et al. 2022). As calculus DNA provides only preliminary identity affiliations of the contaminated samples (Lisman et al. 2023), it can be more useful than bones towards identification strategies of such challenged human remains. Human DNA embedded in calculus hydroxyapatite is comparatively well preserved and can be used as a potential investigative tool for both forensic as well as microbiological research purposes (Higgins and Austin 2013). A minimal 20 mg of dental calculus is ideally collected, though higher amounts of calculus may provide better DNA amplification results (Lisman et al. 2023). The Next Generation Sequencing (NGS) strategies like Amplicon and Shotgun Metagenomics have been used to characterize oral microbiome from the collected dental calculus deposits. Amplicon sequencing of microbial DNA is done to focus on one or more of the nine variable regions of the 16S rRNA gene to characterize the taxonomic structure and diversity of the extracted microbiome (Cappellini et al. 2014).

Mann et al. (2018) reported that DNA obtained from dental calculus is consistently more abundant and less contaminated than DNA extracted from dentin. Most of the DNA obtained from calculus is microbial in origin, derived from the entrapped oral microbial communities in it. The bonafide microbial taxa can be profiled and compared from the identification of microbiomes in ancient or historical dental calculus samples (Belstrom et al. 2018; Weiß et al. 2020). Dagli et al. (2015) identified dental calculus as one of the rich sources of ancient microbial DNA from the microbial colonies entrapped in it and thus, highlighted the implications

of ancient DNA research in palaeo-microbiology. Similarly, calculus can be an alternate source of mtDNA for biological profiling of unknown human skeletal remains older than 1,000 years, which tell us about some benefits and limitations of DNA research from calculus (Black et al. 2011). Adler et al. (2013) sequenced and amplified the 16S rRNA gene of oral microbiota from 5,500 BCE–1,600 CE old dental calculus samples, using the next-generation sequencing technique. Singh and Goel (2017) collected dental calculus from lingual surfaces of mandibular incisors from 20 dental students of Moradabad (UP) and found that majority of dental calculus samples yielded DNA ranging from 21 to 37 $\mu\text{g/ml}$ (mean quantity of 23.5 $\mu\text{g/ml}$). The extraction and sequencing of ancient microbial DNA and its comparison with contemporary microbial strains has significant relevance to practice of modern medicine and dentistry for understanding host-microbiome interactions (Akcalı and Lang 2018). As human oral microbiota is both culture and geography specific, the genetic mutations in ancient microbial DNA help in tracking human migrations that happened in past populations, highlighting the potential ability of microbiota DNA as genetic signal of cultural affinity of pre-historic populations (Dominguez-Bello and Blaser 2011; Eisenhofer et al. 2019). Changes in human oral microbiome signify changes in dietary patterns over time, indicating a marked change in oral pathologies and dental calculus deposition.

Challenges, future probabilities and implications

In most forensic situations, highly degraded and contaminated or challenged samples (containing PCR inhibitors) containing low amounts of DNA are retrieved

for identification purposes which presents a very tough and challenging task for extraction of DNA; dental calculus is one of such materials. The concentration of DNA isolated from calculus does not significantly differ from that obtained from teeth. Dental DNA is comparatively more degraded than the undamaged DNA extracted from calculus, so calculus DNA is more stable and less challenged than dentin DNA (Lisman et al. 2023). However, calculus DNA is more fragmented and shorter in length, it is merely sufficient to obtain a putative profile of an individual; suitable enough, at least, for preliminary forensic identification strategies (Lisman et al. 2023). The biggest challenge for geographic identification of vegetal or faunal inclusions in calculus is the lack of comprehensive reference libraries, and alteration of geolocation signatures due to travel, illness and diet.

Secondly, very limited literature is available about the quantification of human DNA content in dental calculus for human identification purposes. The quantity of collected dental calculus (ideally 20 mg) is crucial as significantly higher amounts of calculus yield better DNA amplification results (Lisman et al. 2023). The benefits and limitations of using dental calculus as an alternate source for DNA should be weighed prior to suggesting its efficacy for forensic purposes. Calculus offers an indirect method of DNA analysis that is more closely linked to a specific individual than other sources which are used for indirect DNA analysis (Black et al. 2011).

Research involving higher numbers of jaw fragments is certainly needed to authenticate the forensic utility of dental calculus for forensic identification purposes. The pioneer research work utilizing the 'to-be-thrown' calculus will cer-

tainly help future forensic, anthropology, public health, and dentistry experts as an important biological material towards establishing identity, geolocality and exposure to heavy metals, and drug abuse of an unknown individual. Forensic anthropologists and geneticists should explore collaborative research possibilities in the field to explore the full potential of dental calculus as an adjuvant and non-invasive evidentiary material towards forensic human identification (Modi et al. 2020). Though the prospect of dental calculus as a biomaterial has yet to be rigorously and systematically scrutinized (Mann et al. 2018), it is expected to help forensic anthropologists for provenance of unknown human remains retrieved from forensic scenarios, even when only some teeth are retrieved from such contexts.

Conclusions

Calculus finds its extensive utility in forensic and archaeological reconstructions such as assessing host-oral microbiota interactions, the microbial health and oral hygiene of past individuals, screen out their dietary status, food diversity, exposures to heavy metal and drug of abuse, and, lastly, to estimate the geographical and occupational affiliations of past individuals. Dental calculus houses a multitude of diverse biomolecules than provide information about diet, health, culture, behavior, ancestry and identity of past populations (Wright et al. 2021). Dental calculus entraps cellular or tissue fragments, past human oral microbiota that can provide deep insights into decedents' lifestyle, disease status, occupational habits, possible geolocation, environmental conditions and the cultural affiliation of an individual; thus presenting a more comprehensive ante-mortem

profile of the unknown individuals (past or contemporary). Microbial forensics has significantly contributed as a useful tool in medicine, bioterrorism, biosecurity, food trade and human identifications (Castro and de Ungria 2022).

Very limited published research work is available about the extraction and analysis of human DNA content from dental calculus for forensic anthropological purposes, thus the anthropological and archaeological research involving dental calculus is still in its nascent stage; so comprehensive research in different population groups is needed for fruitful research avenues and utilization of DCD as an alternative material for ancient genomics research. Anthropological insights into ancient population dynamics, migrations, social stratification, oral health and environmental adaptations can be unraveled from the complex biomolecular composition of dental calculus. Studying DNA from prehistoric bacteria or viruses may lead to the discovery of techniques and approaches to fight contemporary strains/forms of such infectious agents. Recent studies have necessitated that for ancient DNA analysis of current microbial diseases, information about the past genotypic and phenotypic signatures of the responsible bacteria needs to be collected. Thus, crucial knowledge about the past oral microbiome may also help medical professionals and physicians use ancient microbial DNA signatures to track/identify such infectious agents in modern day populations.

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Conflict of interest

The authors have no conflicts of interest to declare.

Author contributions

JSS conceptualized and validated the idea and framework/outline of the manuscript, designed the methodology, scrutinized and finalized the relevant literature on the topic, wrote the rough and final drafts of the manuscript and revised its final version; ST reviewed the final manuscript, edited its English language and generated one figure.

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