Advances and perspectives of dental calculus in archaeological and paleontological research

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INTRODUCTION

In archaeological and paleontological research, methodological approaches and investigative priorities have undergone continuous evolution. Early research primarily relied on fossilized skeletal remains to infer morphological characteristics and behavioral patterns of ancient organisms. As scientific inquiry progressed, scholars increasingly pursued microscopic evidence capable of revealing intricate details about the life and evolutionary pathways of these organisms. This resulted in the emergence of dental calculus analysis as an innovative research frontier.

Dental calculus forms through the mineralization of food residues, oral microbiota, and salivary secretions on tooth surfaces.[1] This calcified deposit provides unique insights into the primary interface of the digestive system, preserving detailed records of oral ecology and food processing mechanisms. As illustrated in Figure 1, dental calculus constitutes a remarkably rich repository containing microbial communities, parasitic remains, botanical microremains (including starch grains and phytoliths), along with preserved proteins and ancient DNA. Its principal scientific value resides in providing direct evidence of paleodietary patterns and ancestral oral microbiomes - information largely unattainable through conventional analytical approaches. The central research question framing this investigation asks: "How can systematic analysis of dental calculus enhance the precision with which we reconstruct dietary behaviors and evolutionary relationships in ancient organisms?" Understanding these aspects proves critical for elucidating ecological adaptations and evolutionary trajectories. This paper aims to critically evaluate current applications of dental calculus analysis across archaeological and paleontological contexts while assessing its future potential, thereby underscoring its unique contribution to evolutionary studies.

CURRENT STATUS OF DENTAL CALCULUS RESEARCH

Historical Development and Main Achievements

Since its recognition as a critical paleodietary archive in the 1970s, dental calculus has undergone significant methodological refinement. Early research paradigms prioritized the identification of microfossils, particularly starch granules and phytoliths, which revolutionized our understanding of prehistoric subsistence strategies by providing direct evidence of plant consumption patterns in early hominins and fauna. This foundational work established dental calculus as an indispensable repository of biocultural information. Recent technological advancements have dramatically expanded analytical capabilities, with current methodologies incorporating stable isotope analysis, paleoproteomics, metagenomic sequencing, and ancient DNA reconstruction. These cutting-edge approaches enable multi-proxy investigations that contextualize dietary practices within their broader ecological landscapes and sociocultural frameworks. By synergistically characterizing organic residues, microbial communities, and biomolecular signatures preserved in calcified deposits, researchers can now reconstruct nuanced dietary profiles while elucidating coevolutionary relationships between ancient organisms and their environments. Such comprehensive analyses not only refine historical interpretations but also provide critical insights into adaptive mechanisms and evolutionary trajectories across geological timescales.

Application in Archaeology

The analysis of dental calculus has become instrumental in reconstructing dietary adaptations during early hominid evolution. In *Australopithecus sediba*, integrative studies combining calculus composition with enamel stable isotope ratios and occlusal microwear topography demonstrate preferential consumption of C3 plant resources.[2] This dietary specialization correlates with Late Pliocene ecosystems dominated by C3 vegetation, suggesting that floral biogeography exerted selective pressures on the species' ranging patterns and resource exploitation strategies. Consequently, these ecological constraints likely drove morphological adjustments in masticatory apparatuses and foraging-related behavioral adaptations.[2] Notably, *A. sediba*'s woodland-based subsistence practices exhibit greater phytodiversity than contemporaneous African hominins, challenging previous assumptions about dietary homogeneity in early hominids. Such discoveries not only redefine the spectrum of ancestral food procurement strategies but also emphasize the critical role of environmental drivers in shaping hominin survival mechanisms and evolutionary trajectories.

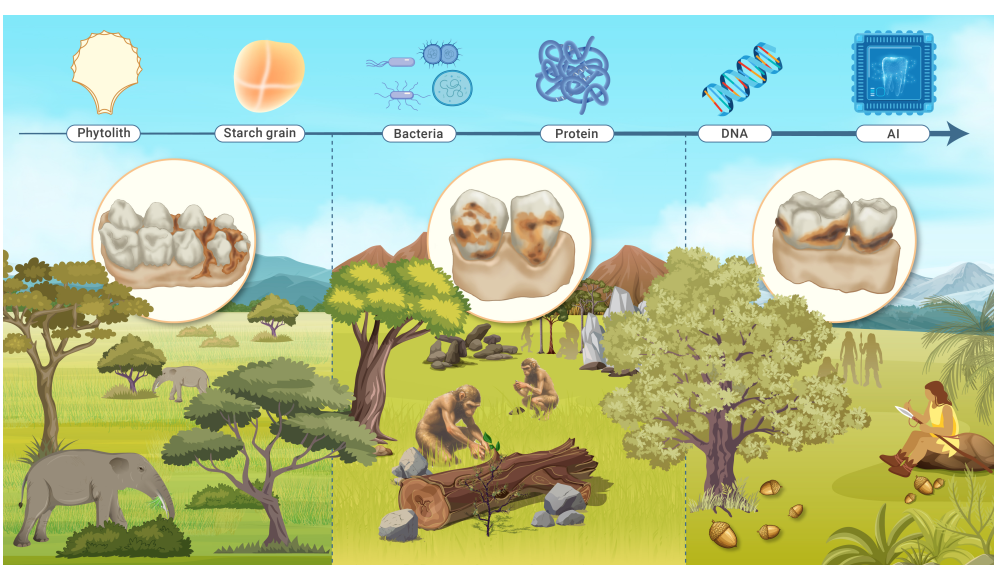

Figure 1. The Ancient World in Dental Calculus

Comparative analyses extend these insights to East Asian contexts. Dental calculus from late Middle Pleistocene *Homo* specimens at Fuyan Cave (Daoxian, Hunan) has yielded diagnostically altered starch granules alongside coniferous phytoliths and parallel striations on enamel surfaces.[3] The striation patterns show remarkable congruence with those documented in Neanderthal dentitions, prompting scholarly debates about potential techno-cultural transmission mechanisms. These micromorphological convergences may indicate cross-regional dissemination of oral processing behaviors during Pleistocene population dispersals. Current hypotheses posit that intergroup contacts facilitated the transfer of subsistence techniques and cultural practices, evidenced by shared material signatures in dental calculus. Systematic investigation of such parallels offers unprecedented opportunities to trace the spatiotemporal development of hominin behavioral complexity and reconstruct dynamic interaction networks among Pleistocene populations.

Application in Paleontology

Dental calculus also holds significant value in studying ancient animal evolutionary processes. In tracing the ancestral lineage of modern elephants, *Gomphotherium steinheimense* (*G. steinheimense*) serves as a critical transitional species. The analysis of its mineralized dental deposits provides crucial evidence for reconstructing dietary evolution. During the Middle Miocene epoch (~16 million years ago) in Xinjiang's Junggar Basin, where forest ecosystems predominated prior to grassland formation, calculus microfossil evidence unexpectedly demonstrates *G. steinheimense*'s graminoid consumption.[3] This grazing behavior represented an ecological anomaly within its arboreal habitat. When subsequent environmental shifts facilitated grassland expansion, *G. steinheimense*'s preexisting graminivorous adaptation conferred selective advantages, coinciding with measurable morphological modifications in dentition.[4] These dental adaptations enhanced mastication efficiency of fibrous vegetation, establishing an evolutionary foundation for the species' successful radiation into proboscidean ancestors during Neogene environmental fluctuations. This chronologically documented progression substantiates organism-environment coevolutionary mechanisms while underscoring dental calculus's unique capacity to elucidate adaptive dietary strategies and evolutionary pathways in paleontological research.

New applications in Protein Analysis of Dental Calculus

Proteomic interrogation has revolutionized the evidentiary potential of dental calculus, enabling unprecedented access to ancestral dietary and physiological conditions. Endogenous proteins sequestered within mineralized plaque matrices serve as direct biomarkers for reconstructing nutritional intake and host-pathogen interactions. The detection of casein peptides in Neolithic hominin calculus has substantiated early dairying practices across Eurasia, while the identification of cereal glutens has refined chronologies of Neolithic subsistence transitions. Such findings establish proteomics as a transformative methodology capable of recovering nuanced biocultural data often obscured by traditional biomolecular approaches.[5]

Despite its analytical promise, calculus-based proteomics confronts significant technical and interpretive challenges. First, the extraction of biomolecules from highly mineralized matrices demands specialized methodologies to mitigate diagenetic alterations or post-depositional contamination. Second, multi-proxy interpretation frameworks must account for taphonomic biases, host oral ecodynamics, and potential allochthonous protein influx from sediment microbiota. Third, comprehensive reconstruction of paleodietary systems requires synergistic integration with archaeobotanical, zooarchaeological, and isotopic datasets.

FUTURE OUTLOOK

With ongoing technological innovations, dental calculus research in archaeology and paleontology is anticipated to yield transformative breakthroughs. Advancements in microscopic imaging and molecular interrogation techniques will enable unprecedented interrogation of ultrastructural and biomolecular details within calcified dental accretions. Next-generation microscopy platforms achieve nanoscale resolution, permitting clear differentiation of plant tissue morphotypes and dietary particulate classifications within microfossil assemblages. Concurrently, cutting-edge metagenomic sequencing and proteomic profiling allow precise taxonomic identification of microbial consortia while elucidating their metabolic roles in oral microbiome dynamics. These methodological developments collectively enhance our capacity to reconstruct ancestral dietary behaviors, pathogen-host interactions, and biocultural adaptations through deep time.

The integration of artificial intelligence with paleoclimatological and paleoecological methodologies promises transformative advancements in dental calculus research. Climatic systems exert fundamental control over ecosystem dynamics through their regulation of vegetation composition and distribution. Variations in temperature-precipitation regimes drive successional changes in plant communities, thereby modifying the nutritional resource base available to ancient organisms and consequently shaping their dietary adaptations. By synthesizing paleoclimate reconstructions, ecological parameters, and phytogeographic data with biomolecular evidence extracted from dental calculus, machine learning algorithms are capable of processing these multidimensional datasets. Through iterative training, such systems can discern non-linear relationships between specific dietary biomarkers (e.g., starch granules or phytoliths) and climate-mediated ecological shifts, as well as identify covariance patterns between oral microbial profiles and environmental variables. This computational approach enables the detection of non-obvious patterns and correlations that elude conventional manual analysis. Researchers can systematically investigate how environmental parameters - including thermal fluctuations, hydrological cycles, and floral succession - influenced ancestral trophic strategies and nutritional ecology. Such insights deepen our understanding of environmental drivers in evolutionary processes while facilitating the development of refined evolutionary models. These models can simulate phenotypic responses to paleoenvironmental gradients and project potential adaptive trajectories under varying ecological constraints.

CONCLUSION

In conclusion, dental calculus analysis constitutes a vital frontier in archaeological and paleontological investigations. This biomolecular archive uniquely bridges anatomical remains with evolutionary trajectories, providing crucial insights into paleodietary adaptations and biocultural dynamics across deep time. As technological innovations accelerate and multidisciplinary approaches mature, the evidentiary value of dental calculus is poised to assume greater significance in reconstructing life's evolutionary chronicles.

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DECLARATION OF INTERESTS

The author declares no competing interests.