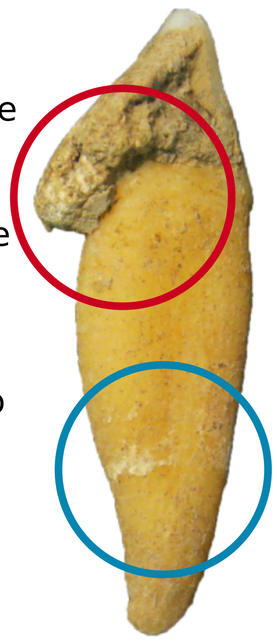
Ancient DNA from archaeological teeth and dental calculus preserves traces of biological processes that occur during an individual's life, and after their death

Allison E. Mann, Susanna Sabin, Kirsten Ziesemer, Åshlid J. Vågene, Krithivasan Sankaranarayanan, Andrew T. Ozga, James Fellows Yates, Christopher Read, Alexander Herbig, Johannes Krause, Menno Hoogland, Corinne Hofman, Kirsten Bos, Christina Warinner

Abstract

Ancient DNA from archaeological remains provides unique insights into past human behavior, health, and evolution. Recent studies have suggested that dental calculus, the mineralized form of dental plaque, may provide a better preservation environment for ancient DNA than other archaeological remains. However, this hypothesis has not been systematically tested. In this study, 48 paired dentin and dental calculus samples from seven archaeological sites representing three continents and wide age range (2920 BCE to 1866 CE) were sequenced using a high-throughput metagenomics approach. We find that dental calculus has a substantially higher DNA yield than dentin from the same individual and maintains a signature of an expected oral microbial community. A small subset of dentin samples (15%), however, retain a minor signature of oral microbes which may indicate oral bacteria participate in decomposition or invade the dentin during life. Finally, human DNA is highly fragmented independent of overall preservation level (mean = 15 bp shorter) in dental calculus but not in dentin. This suggests the mode of incorporation of human DNA in these two substrates is different. We hypothesize this high fragmentation is the result of immune cell activity and bacterial derived nucleases during periodontal disease.



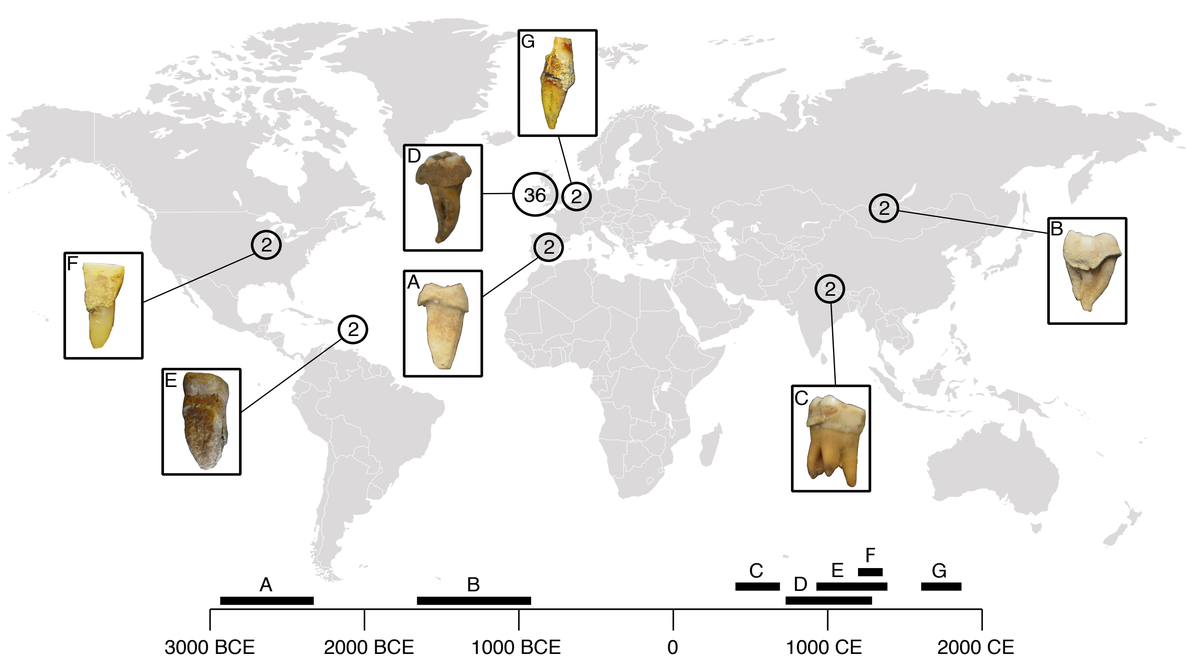


Figure 1: Geographic locations and corresponding age ranges of samples.

Methods

Global dataset sample sites (*Figure 1*):

- A) Camino del Molino, Iberia (n=2)
- B) Hovsgol, Mongolia (n=2)
- C) Samdzong, Nepal (n=2)
- E) Anse a la Gourde, Guadeloupe (n=2)
- F) Norris Farms, IL, USA (n=2)
- G) Middenbeemster, the Netherlands (n=2)

Regional dataset sample site:

- D) Kilteasheen, Ireland (n=36)

All samples were Illumina shotgun sequenced using either 2x100 paired or single end 75 chemistry. Data were quality filtered and mapped to the human genome (hg19) using EAGER[1] and taxonomically binned with MALT[2] against the full NCBI database. Potential source contribution analysis was performed with Sourcetracker[3] and a custom hierarchical categorization procedure[4].

References Cited

[1]Peltzer et al. 2016. Genome Biology 17:60
[2]Vågene et al. 2018. Nature Ecology & Evolution. 2: 520-528
[3]Knights et al. 2011. Nature Methods 8: 761-763
[4]Sabin et al. 2016. Masters Thesis

Acknowledgements

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Results and Discussion

Calculus on average has higher overall DNA yield as compared to dentin (*Figure 2*), though percent human endogenous content is generally lower (*Figure 3*). Human endogenous content in dentin is variable while calculus is comparatively consistent. In addition, human reads recovered from calculus are distinctively fragmented as compared to dentin, independent of overall sample median fragment length (*Figure 4*). It is possible that human DNA is vulnerable to hydrolytic or other damaging processes to the sugar-phosphate backbone during its incorporation into the calculus matrix. Immune cells that produce extracellular chromatin traps are highly active in the oral cavity to combat the formation of plaque; we hypothesize that the incorporation of naked DNA from these traps, as well as bacterial surface nucleases may explain these fragmentation patterns.

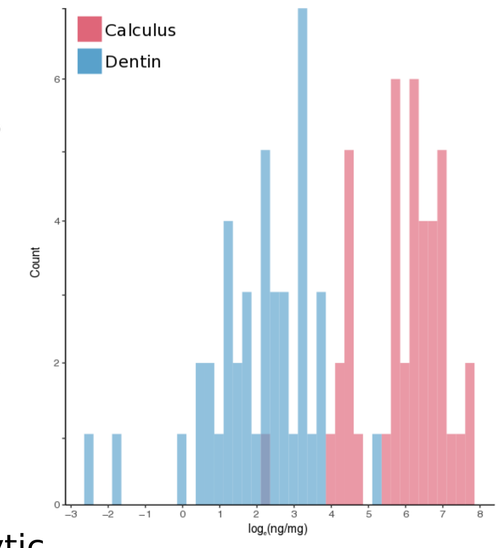

Figure 2: Total DNA yield of dentin versus calculus post extraction

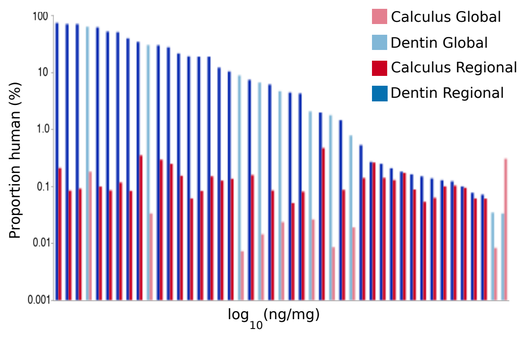

Figure 3: Human endogenous content for all paired calculus and dentin samples

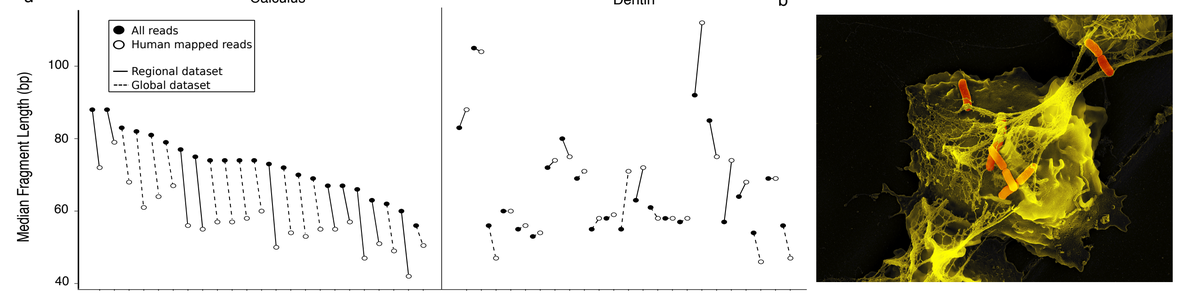

Figure 4: (a) Overall median fragment length versus human median fragment length for all paired end samples. (b) False color electron microscope image of simulated neutrophil netosis and trapped bacteria © Max Planck Institute for Infection Biology

Ancient dental calculus samples retain a strong signature of the oral microbiome with minimal environmental contamination, while dentin samples form a less cohesive group with the majority mapping to exogenous contamination such as skin and soil (*Figure 5*). Surprisingly, a subset of dentin samples preserve a moderate oral signature -- suggesting that oral bacteria participate in the decomposition process, are incorporated during life, or that trace amounts of calculus are still present. This provides one avenue to access individual bacterial genomes in the absence of calculus, providing an avenue to push studies of oral bacteria further into the past where calculus becomes more scare in the archaeological record.These analyses confirms dental calculus as a dependable source of aDNA which can provide insights into past human health and disease states.

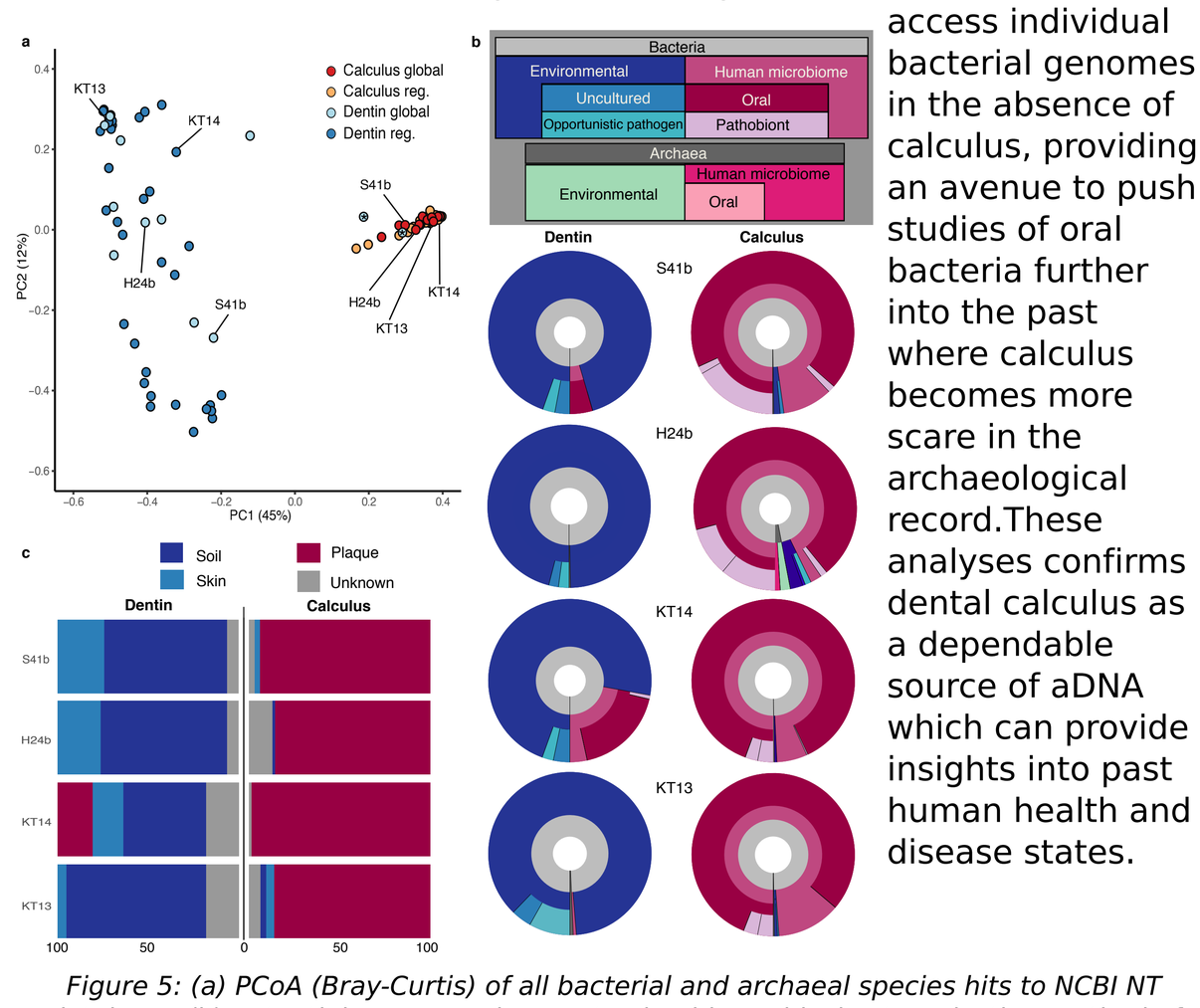

Figure 5: (a) PCoA (Bray-Curtis) of all bacterial and archaeal species hits to NCBI NT database, (b) potential source assignment using hierarchical categorization method of selected samples, (c) potential source contribution of selected samples using SourceTracker[3]