Genetic determination through dental calculus: Promise and hope!

Calculus is a form of hardened dental plaque. Calculus is derived from a Greek word "Calcis," used for various kinds of stones. This spun off many modern words, including "calculate" (use stones for mathematical purposes) and "calculus," which came to be used, in the 18th century, for accidental or incidental mineral buildups in human and animal bodies such as kidney stones and minerals on teeth. Dental calculus is formed by precipitation of minerals from saliva and gingival crevicular fluid in plaque on the teeth. This process of precipitation kills the bacterial cells within dental plaque, but the rough and hardened surface that is formed provides an ideal surface for further plaque formation. This leads to calculus buildup, which compromises the health of the gingiva. Calculus can form both along the gum line, where it is referred to as supragingival and within the narrow sulcus that exists between the teeth and the gingiva, where it is referred to as subgingival calculus.

Calculus is composed of both inorganic (mineral) and organic (cellular and extracellular matrix) components. The mineral proportion of calculus ranges from approximately 40–60%, depending on its location in the dentition and consists primarily of calcium phosphate crystals organized into four principal mineral phases: Octacalcium phosphate, hydroxyapatite, whitlockite, and brushite. The organic component of calculus is approximately 85% cellular and 15% extracellular matrix. Cell density within dental plaque and calculus is very high, consisting of an estimated 200,000,000 cells per milligram. The cells within calculus are not only primarily bacterial but also include at least one species of archaea (*Methanobrevibacter oralis*) and several species of yeast (e.g. *Candida albicans*). The organic extracellular matrix in calculus consists primarily of proteins and lipids (fatty acids, triglycerides, glycolipids, and phospholipids), as well as extracellular DNA. Trace amounts of host, dietary, and environmental microdebris are also found within calculus including salivary proteins, plant DNA, milk proteins, starch granules, textile fibers, and smoke particles.

Calculus formation is associated with a number of clinical manifestations including bad breath, receding gums, and chronically inflamed gingiva. Brushing and flossing can remove plaque from which calculus forms; however, once formed, it is too hard and firmly attached to be removed with a toothbrush. Calculus buildup can be removed with ultrasonic tools or dental hand instruments (such as a periodontal scaler).

Prof. Christina Warinner, who is an anthropology professor at the University of Oklahoma and the co-director of the Laboratories of Molecular Anthropology and Microbiome Research stated "Although dental calculus had been long noted in archaeological reports, it was largely neglected and often discarded. It was widely believed to be virtually abiotic, containing no DNA or proteins, and until very recently, the only real interest in it was to use it to search for chance entrapped plant microfossils such as pollen, starch granules, and phytoliths." During the nascent stages of Prof. Christina Warinner's career in the field of paleogenomics, she took a risk. Instead of utilizing conventional techniques to extract ancient DNA – which often requires destruction of fossil specimens – she took an alternate route and investigated the potential of calcified dental plaque from fossil teeth. She had her doubts, but her curiosity was rewarded and led to, in her own words, "a biomolecular treasure hiding in plain sight." In addition to microbial DNA, Warinner and colleagues found trace amounts of human DNA stored in the dental calculus. It was enough to stoke the flames of knowledge.

Warinner *et al*. recently published their research in American Journal of Physical Anthropology. Using capture enrichment

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technology, the team, which included collaborators from Arizona State University and Pennsylvania State University, extracted DNA from six individuals from Illinois' 700-year-old Norris Farms No. 36 cemetery, which was used by the Oneota, a Native American archaeological culture that rose to prominence during AD 1000–1650, but declined sharply following European contact. When collecting dental calculus, they used same tools as a dental hygienist and essentially gave a very belated tooth cleaning to the ancient individuals. Full mitogenomes were compiled for all six individuals including three who had previously tested negative for DNA preservation in bone samples.

Prof. Warinner also stated that "Dental calculus contains more DNA than any other known archaeological material." Archaeological bone typically contains <2 ng of DNA per milligram of tissue. By contrast, dental calculus typically contains more than 40 ng of DNA per milligram of tissue, and we have even measured samples that contain more than 500 ng/mg of tissue. No other material in the archaeological record contains so much DNA. Further, the samples obtained from dental calculus contain DNA not only from the human but also the microbiome and the diet. Armed with this new technique, anthropologists and archaeologists have a new avenue to acquire mitochondrial DNA, the sequences of which can help them trace the history of the human species and provide glimpses at population movements.

Although dental calculus preserves alongside skeletal remains but it is not actually a human tissue. Dental calculus acquires human DNA and proteins passively, primarily through the saliva and other host secretions. Once mineralized within dental calculus, human DNA, and proteins can preserve for thousands of years. Dental calculus thus serves as an important nonskeletal reservoir of ancient human DNA.

Conventional techniques for recovering ancient human DNA typically require the destruction of bone or tooth tissue during analysis, and this has been a cause of concern for many native and indigenous communities. Dental calculus represents an important alternative source of ancient DNA that does not damage or disturb the integrity of skeletal remains. In addition, because dental calculus is the richest known source of DNA in the archaeological record, it presents unique opportunities for investigating archaeological sites with preservation challenges.

Dental calculus may enable researchers to retrieve ancient DNA from samples where bone or other biological tissues are too degraded for analysis. This is particularly exciting to those of us who work in tropical or extremely old contexts, where traditional sources of DNA may be poorly preserved or even nonexistent. The demonstration that whole mitochondrial genomes can be reconstructed from small samples of dental calculus represents an important technological advancement for paleogenomic investigations in prehistoric North America and other regions where destructive analysis of skeletal remains is difficult or controversial.

Using advanced sequencing technologies, anthropologists demonstrate that human DNA can be significantly enriched from dental calculus (calcified dental plaque) enabling the reconstruction of whole mitochondrial genomes for maternal ancestry analysis – an alternative to skeletal remains in ancient DNA investigations of human ancestry. We can hope that this research on dental calculus from the Norris Farms site acts as the first step toward future paleogenomic investigations.

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