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Pulling teeth from history

DNA from ancient teeth can help to yield information about our ancestors' health, diet and diseases

Philip Hunter

Molecular biology has made considerable contributions to the study of human history from the earliest days of our species, when our ancestors began to walk on the savannah, to the dawn of civilization and the more recent history of mankind. The analysis of contemporary mitochondrial DNA, for example, has told us that *Homo sapiens* first appeared in Africa; the sequencing of the Neanderthal genome has revealed that *Homo sapiens* and *Homo neanderthalensis* lived along each other and interbred before the latter disappeared; last year, the analysis of DNA extracted from a skeleton found under a parking lot in Leicester, UK, showed that it was the remains of King Richard III.

Although the advent of next-generation sequencing and advances in bioinformatics have boosted research using ancient DNA, a major hurdle has always been the quantity and quality of the DNA itself: DNA molecules quickly degrade over time into smaller fragments, and microbial contamination makes it challenging to identify human fragments within a sample. Intriguingly, teeth have proven to be an excellent source of high-quality ancient DNA, yielding insights into the evolution of the human diet, disease and immunity since the onset of agriculture some 10,000 years ago. Dental calculus has been the most complete source of historical sequence data, especially for probing oral microbiome populations. At the same time, dental pulp—the connective tissue at the centre of teeth—has been an important source of information about ancient diseases.

Archaeologist Keith Dobney, from the University of Aberdeen in the UK, and his colleagues first identified the potential of dental calculus as a source of information about past diets and microbial populations long before the advent of ancient DNA studies [1]. Now, almost 30 years later, Dobney is among those at the forefront of the revolution in ancient DNA analysis. "It appears that dental calculus is fantastically well preserved and it's unique", he said. "Unfortunately, it only grows in the teeth, which means you're looking at stuff in the respiratory tract and oral environment".

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The origin of dental calculus is the plaque that forms as bacteria accumulate as biofilms on the surface of teeth. This biofilm then mineralizes in the presence of calcium and phosphate ions in the saliva, preserving not just the microbes but also debris from food and respiration, including DNA and proteins from host immune molecules. This all becomes enmeshed in this mineral matrix and is protected from subsequent contamination or invasion by exogenous micro-organisms. The dental calculus can be dated with high accuracy and it includes a vast number of biomolecules from viruses, micro-organisms and the human host, which enables simultaneous investigation of pathogen activity, host immunity and diet. It thus allows archaeologists to investigate how major changes in diet or the human environment—such as the advent of agriculture or the green revolution and food processing during the 20th century—impact on the oral microbiome and thereby on human health.

One of the main findings so far, reported by Dobney and his collaborators, has been the confirmation that the introduction of farming had a huge impact on the oral microbiome [2]. "It is quite clear there is this conserved human microbiome which massively changes the moment we start to farm", Dobney said. The effect was to diminish the diversity of bacterial species and strains in the mouth, coinciding with significant changes in human health. Agriculture enabled denser populations and thereby the emergence of modern civilization, by freeing labour from finding or preparing food. But greater population densities, combined with close proximity to farm and domestic animals, also led to the emergence of diseases such as measles and smallpox.

The stage for more in-depth research was set by a seminal paper from Christina Warinner and colleagues, which went further in extracting the genomic information of pathogens preserved in dental calculus and describes significant advances in coping with DNA degradation [3]. Warinner and her co-authors showed that ancient DNA and proteins could be recovered from dental calculus in both unprecedented quantity and fidelity, compared with other sources. "The sheer amount of DNA preserved within dental calculus was quite a surprise, orders of magnitude more than from bone or dentine from the same individual", said Warinner, who is a Research Affiliate of the Molecular Research Group at the University of Zurich's Centre for Evolutionary Medicine and part of the microbiome research group at the University of Oklahoma, USA. "Another great unexpected bonus was the extent of

Freelance journalist, London, UK. E-mail: ph@philiphunter.com
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the proteomics data pertaining to human cell types. We did not expect to have such high resolution that we could not only identify a wide range of human proteins, but could even identify the specific human cell types that had been involved in the immune response". This was in part due to the quality of samples preserved in dental plaque, but also the result of advances in bioinformatics tools and algorithms to assist with data analysis. In cases where no software tools were available for the required analysis, the team developed their own.

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One of the major challenges is determining what is known as degradation bias. The deterioration of nucleic acids means that DNA sequences recovered from dental calculus are increasingly fragmented and require sophisticated algorithms to reconstruct. The problem is that the rates of deterioration differ among the various species within the microbiome, which can lead to bias when estimating relative quantities. "Diversity estimates, for example, are highly sensitive to alteration as microbiomes degrade and accumulate miscoding lesions or simply become too fragmented to amplify the microbial community without bias or skew", Warinner explained. "We are currently working on additional methods for identifying deterioration bias in ancient microbiome samples".

One important question will be the relationship between oral microbiome diversity and human health, particularly in recent times, as it has clear relevance for today's diseases. "The industrial revolution gave another push in the direction of declining diversity in our oral microbiome and looking at our modern western samples it is probably still getting worse today", Dobney said. He went on to speculate that this could be contributing to a number of diseases that have become more prevalent in developed countries, especially type 2 diabetes and other metabolic or inflammatory-related disorders, although he cautioned that further research was needed to establish this link.

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Warinner commented that the meaning of the term pathogenic in the context of the oral microbiome has evolved as well. "With respect to the microbiome as a whole, the word 'dysbiosis' is more commonly applied", she said. "Microbiomes are thought to generally evolve in mutualistic symbiosis with their hosts, but drastic shifts in behaviour or diet can cause microbial imbalances and initiate a state of dysbiosis, or dys-symbiosis. This is generally described as a state of microbial imbalance in which the formerly mutualistic relationship between microbes and the host has turned harmful. Dental caries and periodontitis (gum disease) have been described as diseases of oral dysbiosis".

Although events such as the introduction of farming leave a clear signature on the oral microbiome, the specific casual factors need to be determined. "The classical hypothesis has been that an increase in carbohydrates, that is wheat, barley or rice, impacted the microbiome, but in reality, there are lots of alternative hypotheses", commented Laura Weyrich, a Postdoctoral Research Associate at the Australian Centre for Ancient DNA, University of Adelaide, who collaborated with Dobney and others on the dental calculus analysis. "For example, European farmers could be individuals that have migrated from another part of the world and brought a foreign microbiome with them. Introduction of milk could have changed the microbiome, or simply hygiene and exposure to lots of other individuals and disease could have drastically impacted the microbiome. There were a lot of cultural changes during the Neolithic (farming) revolution that we have to keep in mind. Things like population density, disease, and cultural practices could have also easily impacted the microbiome".

Farming did not arrive in a single event, but at different times and in different ways with a range of dietary outcomes. In many cases, farming and hunter gathering coexisted, raising the question of whether this overlap itself could be detected in the bacterial DNA from dental calculus. "We believe we have evidence of this in some of our ancient calculus specimens", Weyrich said. "We have a hunter-gatherer living almost contemporaneously with early Neolithic farmers in Europe, and we see that this individual carries microorganisms from both the farmers and the hunter-gatherers. Whether or not this is diet induced, or simply exposure to two different human groups, we don't know yet".

Dental calculus may have emerged as the best source of ancient biomolecules, but other sources are also important, as there are wide variations in microbial populations throughout the body of even a small animal. "Even within our own mouth, the bacterial communities on the surface of the tongue are distinct from those on the roof of our mouth, despite the fact that the two surfaces are in direct contact several thousand times a day", Warinner noted.

The tooth, either via plaque or dental pulp, has also been the source of DNA sequence data for investigating past diseases, because the DNA remains protected against both contamination and invasion from exogenous sources. Pulp has been the major source for the analysis of specific bacterial DNA information, because pathogens are far more prevalent in the bloodstream than in the mouth. One of the earliest investigations concerned the Justianic Plague (ca. AD 541–542), a pandemic caused by *Yersinia pestis* that afflicted the Eastern Roman Empire, especially its capital, Constantinople (now Istanbul), the Sassanid Empire and Mediterranean port cities. The plague is estimated to have killed 30–50 million people across Europe and Asia. *Yersinia pestis* causes both Bubonic Plague and the more virulent Pneumatic Plague, with the difference being that the former is localized to the lymphatic system, while the latter affects the respiratory system and spreads more rapidly through airborne droplets.

The most recent study involving a multidisciplinary team from institutions in Germany, the USA, Canada and Australia found that the Justinian Plague, like the Black Death that swept through Europe in the

mid-14th century, involved the more deadly pneumonic form of the plague, but was caused by a different strain of the pathogen [4]. The analysis was carried out on DNA fragments extracted from tooth pulp from two victims of the Justinian Plague, found buried in a gravesite in a small cemetery in the German town of Aschheim. The research team believe the victims died in the latter stages of the epidemic when it had reached southern Bavaria, probably between AD 541 and 543.

The DNA analysis itself does not indicate whether the two victims suffered from pneumonic rather than bubonic plague, since the pathogen is the same, but the source of the DNA, the tooth pulp, does indicate which version of the plague the victims had, according to Holger Scholz, a senior scientist at the Bundeswehr Institute of Microbiology in Munich and one of the study authors. "Bubonic plague is a local infection, caused by the bite of an infected flea, which leads to the enlargement of the lymph nodes (buboes), especially in the armpit and groin", he said. "Since we have isolated the DNA from tooth pulpa, it must have been septicaemic (generalized infection). Only in the case of a septicaemic infection is *Y. pestis* in the blood stream. So it must have been pneumonic plague, either primary pneumonic plague or secondary with generalized infection". Primary pneumonic plague occurs when the lungs are directly affected, while secondary is when the pathogen first invades the blood to become systemic and reaches the respiratory system afterwards. The study also resolved other questions about the Justianic Plague, such as whether it originated in Africa or Asia. "We concluded that the Justianic Plague, like the Black Death and the third pandemic, originated from Asia because all *Y. pestis* strains analysed so far around the position of the Justianic strain came from Asia", Scholz explained.

Meanwhile, more light has been shed on the Black Death (ca. 1346–1353), the second *Y. pestis* pandemic, as a result of London's Crossrail project to construct a high-speed underground rail link beneath the city. During excavations early in 2013, 23 skeletons that all appeared to be victims of the Black Death were discovered 2.5 metres below the road that surrounds gardens in London's Charterhouse Square. The depth of the burials and dating of surrounding pottery indicated these victims died around 1348, rather than in the later outbreak starting in 1361. "These human remains are the first to be excavated from this cemetery and not burials associated with the later development of Charterhouse on the site after AD 1370", said Lucy Whittingham, Post-Excavation Manager at the Museum of London. "Thus, this was an exciting opportunity to investigate ancient DNA and establish if it could be found in these skeletons and was related to the Black Plague and its European counterpart". Whittingham added that the museum was still working on the initial results for publication. "The results to date indicate that there are four samples which test positive for the presence of *Y. pestis*. These are four different individuals, one of which is definitely from the earliest phase of the excavation and likely to be the 1348 plaque outbreak".

Another important study was conducted at the University of Tübingen in Germany with a particular focus on the legacy of ancient *Y. pestis* pandemics on the modern populations of this bacterium [5]. As more data on the contemporary populations has emerged, the team has made significant progress in probing how the bacterium has evolved. "Later in 2012, after my paper was published, over 100 modern pestis genomes were made publicly available, and an additional ancient one was published earlier this year", said Kirsten Bos, the first author of the Tübingen study. "We're now working at reanalysing our data in the context of these new strains".

One question Bos is pursuing is why plague epidemics petered out around 260 years ago, even though the ancient strains identified so far from plague victims are genetically very similar to current strains. "Plague epidemics seem to have occurred at a regular frequency in Europe starting in at least AD 540 and persisting until about the mid-18th century, when they abruptly stopped", Bos said. "One possible reason is that successive selection events occurred in human immunity genes. In other words, the pathogen changed very little, but humans adapted to better deal with *Y. pestis* infections. We can therefore look for changes in allele frequencies over time in the human genome to investigate host-pathogen coevolution". The sudden cessation in plagues could also be explained by changes in rodents that are important vectors of the disease in humans. "In this respect, the reasons for differences in rates of plague infections over time may have more to do with the dynamics of the vector species as opposed to humans themselves", Bos said. "Future research will help to address many of the outstanding questions".

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The answers could, as in the case of dental plaque analysis, have real contemporary applications. "Ancient diseases give us some information about natural variation that can occur in pathogens and how they evolve", Bos said. "Knowing how they change and what forms contributed to past pandemics will be valuable in terms of modern disease management and screening". In addition to elucidating the history of infectious diseases in greater detail, there is great therapeutic potential through exploiting knowledge of the evolving relationship between diet, the human microbiome, the immune system and disease.

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