INNER WORKINGS

Ancient teeth reveal clues about microbiome evolution

Jyoti Madhusoodanan, *Science Writer*

Christina Warinner often spends the better part of her day rubbing toothbrushes and dental scrapers against filthy jaws. But she is not a dental hygienist. A molecular anthropologist at the University of Oklahoma, Warinner chips away at ancient teeth to collect dental calculus, the thick chalky layer that hygienists scour away.

These yellowing flakes, Warinner has found, trap not only microscopic food remnants, pollen grains, and proteins, but also ancient DNA, both human and microbial. Warinner's primary aim: understanding the role and the evolution of ancient microbiomes. What's considered damaging debris at the dentist's office is, for Warinner, an invaluable bacterial treasure trove that could link the microbiomes of ancient peoples to our own, revealing secrets about disease and the health of modern-day humans.

"We know almost nothing about the evolution of the human microbiome," says Warinner. "We have dramatically altered our lifestyles over the last several centuries; how has this affected our microbes?"

Warinner aims to address that question by comparing the microbial inhabitants of our bodies across space, time, and lifestyles. Studying the microbiomes of people living in different parts of the world, ranging from Tanzanian hunter-gatherer groups to city-dwellers in Houston, reveals how different lifestyles correlate with differences in our microbiomes. For example, studies have suggested that indigenous populations have greater diversity in their microflora than those from industrialized societies (1, 2).

Researchers retrieved dental calculus from skeletal remains such as this ~1,500-year-old jaw, excavated in 2012 at a site in Samdzong, Nepal. Image courtesy of Christina Warinner (University of Oklahoma, Norman, OK).

Curious Coating

Everyone's teeth are coated with a sticky biofilm, known as dental plaque, that's made of microbes and proteins. Over time, minerals deposited in plaque harden to form tartar, or dental calculus, trapping bits of food. Early studies of fossilized teeth and calculus focused on these remnants of ancient diets.

But while trying to replicate a study of starch granules embedded in ancient tartar, Warinner noticed a problem. "We were having so much trouble locating the starch granules because all these bacteria were in the way," she says. "I had this Aha! moment: maybe these bacteria are interesting and maybe no one has looked at them yet." She started to try to identify these bacterial nuggets.

Warinner's initial experiments to extract microbial DNA failed, however. Most protocols to study ancient DNA are designed for trace amounts of samples, and calculus simply carried too much. "We went from thinking it had no DNA to considering it the richest source," she says.

Combining the methods used by ancient-DNA researchers and those of modern microbiome studies led to the first study describing the contents of our ancestors' mouths (3). In comparing microbiomes from 1,000-year-old remains at a medieval monastic site in Germany to nine living people with known dental histories, the study was the first to report that calculus did in fact harbor ancient microbial DNA. Warinner and her colleagues found rich evidence that strains linked to periodontal disease today, such as *Tannerella forsythia* and other "red complex" microbes, were present in the ancient samples, thus confirming a long association between these pathogens and human oral health.

Fossilized feces, known as coprolites, which Warinner also studies, have rich bacteria stores, but those remnants can decompose quickly. Calculus consists of the same hard, calcium-based deposits that form bones and teeth. Boosting its preservation capacity, calculus mineralizes even in living organisms to form large, dense crystals rather than the smaller structures found in bones and within teeth. "It essentially fossilizes while

 www.pnas.org/cgi/doi/10.1073/pnas.1606592113

you're still alive, so it's protected from the decomposition process when an organism dies," says Warinner. "When we've analyzed both dentin and calculus from the same tooth, we've found that the DNA in the calculus seems better preserved." Following a lead from their first report of the microbes in calculus, Warinner and her colleagues found that trace amounts of human DNA present in 700-year-old calculus could even reveal evidence of ancestry. Using samples from a Native American cemetery in Illinois, the researchers successfully recovered human mitochondrial DNA, which is often used to track lineages (4). The study recovered high-resolution sequences for the complete mitochondrial genome of six individuals from the Oneota, a once-prominent Native American culture that declined after European contact.

Detecting Disease

Because the mouth is a gateway into the body, dental calculus also harbors systemic pathogens. "Even if they're not necessarily part of the oral microbiome, they're transient and become entrapped; it offers us a bit of a snapshot on disease exposure," says Camilla Speller, an archaeologist at the University of York. Already, Speller and Warinner have dug up signs of sexually transmitted pathogens, such as *Neisseria gonorrheae*, and gastrointestinal pathogens, possibly from drinking contaminated water, in ~1,000-year-old remains found in Germany.

By comparing data from diverse modern samples with ancestral ones, researchers can start to map microbiome changes among different peoples. "Between these two kinds of evidence, we can begin to understand what microbes everybody had, whether you were a Viking or a hunter-gatherer in Peru," says Jessica Metcalf, an evolutionary biologist at the University of Colorado in Boulder. "And are those missing in urban people today?"

Intriguing comparisons are emerging. Last year, for example, researchers compared the gut microbes of an urban American population to a rural agricultural community in Peru and a Peruvian hunter-gatherer community. The researchers found that both rural groups, like all traditional communities and nonhuman primates studied so far, carried microbes named *Treponemes*, thought to be a symbiotic species lost in industrialized communities (5). Because *Treponemes* and related species metabolize carbohydrates, it's possible that these organisms might persist in populations where high-fiber diets are common.

Considering many different kinds of human hosts—including our ancestors—will help elucidate the links

Scientists collect samples from a tooth found in Chen Chen, an important Tiwanaku site near Moquegua, Peru. It dates to ~1000 CE. Image courtesy of Christina Warinner (University of Oklahoma, Norman, OK).

between microbiomes and human health, says microbiologist David Relman of Stanford University, who studies the microbiomes of the living. The challenge is piecing the information together. Because most studies so far have only found correlations between diet, age, or lifestyle with differences in

> "Ancient microbes are such a huge piece of the puzzle because there are so few ways to understand and reconstruct the microbiomes of pre-antibiotic lifestyles."
>
> —Jessica Metcalf

microbiomes, it's difficult to know how our ancestors' habits were linked to their microbes. "We don't know much about the health of the individuals from whom these ancient samples came, and we do know that the world in which they lived was very different," Relman says, adding that the ancient microbiome data could still provide important insights.

"Ancient microbes are such a huge piece of the puzzle because there are so few ways to understand and reconstruct the microbiomes of pre-antibiotic lifestyles," Metcalf says. "We need that information before we start manipulating the human microbiome for therapeutics."

1 Schnorr SL, et al. (2014) Gut microbiome of the Hadza hunter-gatherers. *Nat Commun* 5:3654.

2 Human Microbiome Project Consortium (2012) Structure, function and diversity of the healthy human microbiome. *Nature* 486(7402):207–214.

3 Warinner C, et al. (2014) Pathogens and host immunity in the ancient human oral cavity. *Nat Genet* 46(4):336–344.

4 Ozga AT, et al. (2016) Successful enrichment and recovery of whole mitochondrial genomes from ancient human dental calculus. *Am J Phys Anthropol*, 10.1002/ajpa.22960.

5 Obregon-Tito AJ, et al. (2015) Subsistence strategies in traditional societies distinguish gut microbiomes. *Nat Commun* 6:6505.