Putting dental calculus under the microscope

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Summary

Dental calculus. This small, hard, inconspicuous substance that forms on the teeth of humans and animals contains a surprising amount of information about our lives. During formation and growth as a living plaque biofilm, it tends to accumulate a wide variety of very small particles, especially bacteria and food debris, from various sources. These sources and the particles they leave behind in our mouth are influenced by activities and biological processes that are unique to us, such as our dietary preferences, oral hygiene practices, genetics, and the environment in which we live. What makes it so interesting to archaeologists is that, following mineralisation, these particles become trapped and well-protected against removal and degradation during hundreds to thousands of years in the ground, preserving a picture of the activities performed by its human host. This picture can be unlocked by archaeologists by extracting and identifying proteins from plants and animals, and genetic material and microremains from plants that were trapped inside the calculus matrix. The major problem—one of the major problems, for there are several—one of the many major problems is that this picture was never a complete picture of a lifetime of activities. Another problem is that it has faded over the years, and some parts of the picture have been completely erased. There are many things influencing what gets trapped inside dental calculus, what gets preserved for all those years until it can be analysed, and how much of that information we can extract

and interpret. We know that these problems exist. We know that they limit our interpretations of past activities. We need to approach these problems more systematically at a fundamental level.

We need to find out more about what exactly is causing external particles to become trapped inside our dental calculus, and be able to quantify exactly how they impact our interpretations of dietary practices from archaeological dental calculus.

My dissertation introduces a potential method for resolving these issues, namely a protocol for growing artificial dental calculus in a lab. Working with a very controlled model of dental calculus in a lab allows me to explore the influence of a wide range of factors that may influence the uptake of particles into dental calculus, and what biases are introduced by these factors as well as the methods we currently use to extract information from archaeological dental calculus. Addressing these fundamental issues and limitations will go a long way towards improving the resolution of our interpretations of past dietary activities. I also explore new ways to extract information from archaeological dental calculus to learn more about our past.

Chapter 1 is a brief introduction into the many uses of archaeological dental calculus to reconstruct the diet of past populations. I also outline the current state of dental calculus research, and some of the problems we are facing. I only briefly describe what dental calculus is, and how it is formed; this is an important concept to understand, since it influences the uptake of food particles, and is influenced by diet. **Chapter 2** provides more detail on the formation and growth of dental plaque, and mineralisation to form dental calculus. Here, I also provide an overview of oral biofilm models to provide some context for the experiments I conducted in my dissertation research using an oral biofilm model

Chapter 3 is the first article, which introduces the oral biofilm model I developed for my research. In this article we also assess the ability of our model to mimic the properties of natural dental calculus in order to justify using model calculus as a proxy for archaeological dental calculus. By characterising the bacteria present in the model calculus, we found that it was indicative of an oral microbiome, though somewhat distinct from the natural calculus we used as a comparison. We also determined the mineral content using Fourier Transform Infrared (FTIR) spectroscopy, which established that the model was primarily made up of carbonate hydroxyapatite, the predominant mineral in natural dental calculus. The second article that forms **Chapter 4** we applied the calculus model to find out what happens when we add a known amount of dietary starch granules to model calculus during formation, and attempt to extract the starches using a common method for extraction of starches from archaeological dental calculus. We were able to validate what previous studies on modern humans and non-human primates have shown, that the quantity of starches that we extract from calculus, is not very representative of the dietary intake. We also discovered that one of the causes for this misrepresentation of the starch record is that large starch granules were being incorporated at a lower rate than smaller granules. This was demonstrated by the fact that potato granules, which are quite a bit larger than wheat, were underrepresented in our extracted counts.

In addition to diving into the causes behind dental calculus limitations, we also sought out to find novel uses for archaeological dental calculus. In **Chapter 5**, the final article in this dissertation, we used a novel method employing ultra high performance liquid chromatography-tandem mass spectrometry (UHPLC-MS/MS) to identify various plant-based alkaloids traditionally used for medication and non-dietary activities, such as nicotine, opioids, and cannabinoids. We were unable to detect opioids and cannabinoids, but we found evidence of alkaloids and metabolites derived from the consumption of tea and/or coffee, as well as nicotine (and its metabolite, cotinine), and salicylic acid, the primary phytohormone in willow bark. We combined these results with the presence of skeletal and dental indicators of disease to find patterns of potential disease management. We were unable to definitively link the presence of these with evidence of disease to justify medicinal activities, but we found some interesting correlations between maxillary sinusitis and markers of tobacco-use and consumption of tea and coffee.

In the final part of the dissertation, **Chapter 6**, I discuss the outcomes of the studies from previous chapters, addressing the oral biofilm model and its implications for archaeological research. I lay out the challenges we need to accept to further our understanding of archaeological dental calculus and how it relates to the dietary activities of the people we study in the past. This includes systematically conducting more fundamental research to understand the mechanisms causing various dietary (and non-dietary) markers to become entrapped in dental calculus.