Report

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Phytoliths can cause tooth wear

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Comparative laboratory sliding wear tests on extracted human molar teeth in artificial saliva with third-body particulates demonstrate that phytoliths can be as effective as silica grit in the abrasion of enamel. A pin-on-disc wear testing configuration is employed, with an extracted molar cusp as a pin on a hard disc antagonist, under loading conditions representative of normal chewing forces. Concentrations and sizes of phytoliths in the wear test media match those of silica particles. Cusp geometries and ensuing abrasion volumes are measured by digital profilometry. The wear data are considered in relation to a debate by evolutionary biologists concerning the relative capacities of intrinsic mineral bodies within plant tissue and exogenous grit in the atmosphere to act as agents of tooth wear in various animal species.

1. Introduction

It has long been recognized that tooth wear in mammals is compounded by the presence of microscopic silaceous particulates in the environment [1–4]. However, the debate has continued over the dominant source of these particulates, whether exogenous silica grit in the atmosphere or opaline phytoliths in plant matter. This is an important issue on two scales: macrowear, the millimetre-scale removal of dental tissue over a tooth height, threatening the long-term survival of herbivores and omnivores that consume silica-rich foods; and micro-wear, the pattern of micrometre-scale pit and scratch markings used to infer dietary histories in fossil remains. One group of authors, employing precision nanoscratch experiments on individual particulates, has argued that phytoliths are ineffective agents of wear [5–7]. Echoing an earlier study [8], they posit that while phytoliths are basically silicate in composition, their hardness is significantly lower than that of exogenous silica particles and even enamel, thus too soft to effect meaningful tooth wear. Another group challenges this assertion by questioning that interpretation and the reliability of hardness tests on micrometre-scale specimens [9,10]. Consequently, there is a need for clarity in the roles of competing particulate types in the broader discussion of tooth wear.

What appears to be missing in the back and forth is systematic macrowear evidence—can phytoliths cause full-scale enamel loss under long-term abrasive conditions? In this report, we present results of sliding wear tests on extracted human molar teeth in an artificial saliva medium, with and without additive phytoliths or silica grit. We use a conventional pin-on-disc wear test arrangement, with the tooth cusp as pin on a flat antagonist plate, under conditions commensurate with normal biting function. Building on previous single-particle experiments [5,11], we use a wear test that enables us to separate the mechanics of particle-on-enamel abrasion from the complex processes of real-life chewing. We demonstrate that phytoliths can indeed cause substantial cuspal wear, comparable to that of silica grit of similar scale. Implications concerning the existing microwear dialogue are considered.

Figure 1. Silica grit particles and phytoliths used in wear test. SEM images illustrate representative particle size and shape variation. Bar charts show distributions of maximum particle dimension. Phytoliths lie in the same size range as silica, but are a little more elongate with sharper corners.

2. Material and methods

Impacted human wisdom teeth extracted from healthy adults (CICOM clinic, Badajoz, Spain) and free of surface damage were cleaned and kept in aqueous solution. Those with a prominent round cusp were selected for testing. Ensuing wear experiments were run within one week of extraction, to minimize any change in properties from ageing. Secondary cusps and roots were machined away from each tooth and the bases ground flat. The prepared teeth were placed in three groups of five for subsequent wear testing in different media.

Phytoliths were obtained by acid extraction from wheat stems and leaves (electronic supplementary material). The moderate temperature acid extraction used in the plant processing is not expected to change the phytolith physical properties [12]. Silica grit was obtained from a commercial source (A. Anglada Suministros Ceramicos, Zaragoza, Spain). Both phytolith and silica batches were sieved to remove particulates greater than 80 µm. Image analysis software (Image-Pro Plus 6.0, Media Cybernetics Inc., Rockville, MD) was used to analyse size distributions of the two particle groups from photographic images, before and after testing. Maximum longitudinal dimensions L and lateral dimensions D were evaluated from some 120 particles/group. Each group of particles was dispersed by sonication for several minutes at a concentration of 0.5 vol% in a test medium of artificial saliva (Lacer S.A., Barcelona, Spain) immediately prior to testing.

Tests were conducted in a pin-on-disc wear machine (Tribometer THT, Anton Paar, Graz, Austria) at room temperature. Each molar cusp was glued onto a pin holder mounted at a distance 3 mm from the machine axis (electronic supplementary material). The molar cusps were placed in contact with a highly polished dental-zirconia disc and immersed in the saliva/saliva + particles medium. A load of 30 N was applied to the specimen holder, and the disc rotated at 32 r.p.m. for 250 min, corresponding to a sliding distance of approximately 150 m. The medium was replenished after each test run.

The molar cusp geometry was mapped by digital optical profilometry (Profilom, Filmetrics, San Diego, CA) before and after wear testing (electronic supplementary material). Measurements were made of initial molar cusp radius R , wear height h and scar cross-section area A . Wear volume was determined as hA (electronic supplementary material). Optical microscopy (Epiphot 300, Nikon, Tokyo, Japan) and scanning electron microscopy (SEM; S-3600, Hitachi, Tokyo, Japan) were used to examine wear tracks on the scar surfaces.

3. Results

SEM images of the silica grit and phytoliths are shown in figure 1, along with distributions of particle dimensions from image analysis. Means and standard deviations were longitudinal dimension $L = 30 \pm 11$ µm and aspect ratio $L/D = 1.5 \pm 0.5$ for silica; $L = 24 \pm 16$ µm and $L/D = 2.2 \pm 1.3$ for phytoliths. The phytoliths are a little smaller and somewhat more elongate, flatter and sharper than the silica, but their longitudinal dimensions lie in the same broad range. The particulates remained intact through the test duration (electronic supplementary material), despite having inflicted substantial wear on the enamel specimens.

Figure 2a shows profilometer traces of a typical molar cusp before and after wear testing, from which cusp radius R , scar height h and area A could be digitally evaluated (electronic supplementary material). Representative wear volumes hA for the entire dataset are plotted on the bar chart in figure 2b, means and standard deviations of five specimens/group. Student t -tests indicate that the differences between tests in media with and without either silica or phytolith additives are significant ($p < 0.05$), but that differences between the two particle types are not ($p > 0.05$) (electronic

Figure 2. Evaluation of wear. (a) Profilometer trace of typical human molar cusp before and after wear testing, showing height h and area A of scar. Vertical axis exaggerated. (b) Bar chart plotting mean and standard deviation of wear volume parameter hA for each group of five specimens in the three test media. (c) Wear scars on specimen tested in saliva medium (S), saliva plus phytoliths (S + P) and saliva plus silica grit (S + S).

supplementary material). Student t -tests on the tabulated cusp radius data R indicate no significant difference in effective tooth size between groups.

Figure 2c compares SEM images of wear facets on the molar pins for saliva (S), saliva plus phytoliths (S + P) and saliva plus silica grit (S + S) media. Whereas the scar in image S shows relatively fine tracks with some surface smearing, those in images S + P and S + S reveal micrometre-scale gouges from individual particle microcontacts, indicative of wear in the severe abrasion region [13].

4. Discussion

The current results on human molar cusps from a conventional tribology test configuration implicate phytoliths as effective abrasive tooth wear agents. Data analysis shows that differences in wear volumes between groups with additive phytolith or silica particles in artificial saliva are *not* statistically significant. However, differences in wear volumes between tests in saliva with and without either particulate kind *are* significantly different. While there is spread in the data, attributable in part to geometrical tooth-to-tooth variations, measured cuspal radii R between the three test groups are statistically similar. SEM examinations of tracks within wear scars visually confirm the contention that phytoliths can indeed effect enamel removal.

The question may be raised as to how demonstrative these data are of natural tooth wear in mammals, especially in the knowledge that wear tests can be variable. Real-world mastication is complicated by the convoluted nature of occlusal contact motion over prolonged time scales in different

species and by a wide range in food properties. Chewing machines can take us closer to reality [14,15], but at some expense in analytical simplicity. The conditions of our basic wear tests are not atypical of tooth function for human molars [16]: normal load of 30 N per cusp, occlusal facet diameter approximately 0.5 mm, simulated saliva medium. Each test corresponds to $150 \text{ m}/0.5 \text{ mm} = 300\,000$ passes (sliding distance/wear scar diameter), i.e. toward a lifetime of mastication in humans. It might be argued that since our tests are run against a hard (zirconia) antagonist, our observations represent a worst-case scenario. However, contact damage in brittle materials is relatively insensitive to the antagonist material, provided the latter is at least as hard as the specimen [17,18]. The mode of material removal described in the current tests, most apparent in figure 2c, indisputably falls into the category of 'severe abrasive macro-wear' [13]. One group of researchers [5] in scratch tests with individual particles attached to a nanoindenter tip have concluded that phytoliths cause only 'rubbing' marks on enamel, with material displacement rather than removal. Their experiments lie in the domain of microwear, with implications in the interpretation of residual markings on fossil teeth. Based on instrumented nanoindentation hardness measurements on individual particles [6,8], Lucas *et al.* conclude that phytoliths are too soft to cause abrasive wear on enamel. Their quoted hardness ranges for typical phytoliths are substantially lower than those for silica grit and marginally lower than those for enamel. Others [10] contest this conclusion, arguing that nanoindentation experiments on such small particulates cannot be considered reliable, and that the hardness values quoted are inconsistent with those from previous workers. In this context, it has been found

from indentation plasticity theory that particles with hardness as low as one third that of another body may still scratch that second body [17], a contention supported in reciprocating sliding microwear tests with soft metal spheres on enamel [11]. Moreover, as acknowledged by Lucas *et al.*, even displaced material becomes susceptible to removal in subsequent, multiple scratch events. Recall that even tests in particle-free saliva, with their relatively fine surface scratches (figure 2c), still produce measurable wear (figure 2b).

This is not to dispute the essential findings of Lucas *et al.*, but simply to point out that their scratch experiments are conducted in a low-load 'sub-threshold' region, where contact deformation is exclusively plastic, whereas our current tests are conducted in a comparatively high-load 'post-threshold' region, where microcracks may coalesce and facilitate the kind of abrasive wear seen in figure 2c [19]. The onset of microcracking may be aggravated by sharp phytolith corners [20,21] and ensuing coalescence to be cumulative in multiple sliding contacts [22]. Extensive damage of this kind is most likely to prevail in prolonged high-load occlusal mastication, as with some mammals who wear their teeth down to the gumline [23]. Whether endogenous phytoliths or exogenous grit dominates in the wear of mammalian dentition in nature comes down to diet and environment—teasing apart these competing sources has proved challenging. Several studies on specific animals, in the wild or captive and with different

particulate geometries, make the case for biogenic silicates in vegetable matter as a primary source of wear [24–30]; while others, especially in relation to hypsodont grazers, implicate soil grit [31–33]. It is plausible that encounters with post-threshold abrasive contacts of any complexion are sufficiently sparse as to allow plastic flow to dominate microwear patterns in some animals, but at the same time sufficiently frequent to exacerbate abrasive macrowear over extended lifetimes in others. Regardless, the evidence is compelling that phytoliths are capable of producing tooth macrowear, if only in severe conditions, comparable in degree to that of silica grit.

Ethics. Permission to use human dental tissue was granted by the Bioethics Committee of the Universidad de Extremadura under approval ID nos. 19//2012 and 213//2019.

Data accessibility. Data are included as electronic supplementary material.

Authors' contributions. F.R.R. and O.B.L. conducted the research. A.G.H. supplied materials. B.R.L. and P.J.C. wrote the paper with contributions from O.B.L. and A.G.H.

Competing interests. We declare we have no competing interests.

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