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Citation

Rodriguez-Rojas, F., Borrero-Lopez, O., Constantino, P. J., Henry, A. G., & Lawn, B. R. (2020). Phytoliths can cause tooth wear. *Journal Of The Royal Society Interface*, 17(172). doi:10.1098/rsif.2020.0613

Version: Accepted Manuscript

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Downloaded from: <https://hdl.handle.net/1887/3221163>

Note: To cite this publication please use the final published version (if applicable).

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-disk wear testing configuration is employed, with an extracted molar cusp as pin on a hard disk 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.

Key Words: phytoliths, silica grit, wear, tooth enamel

35 **1. INTRODUCTION**

36

37 It has long been recognized that tooth wear in mammals is compounded by the presence of
38 microscopic silaceous **particulates (1-4)**. However, debate has continued over the dominant
39 source of these particulates, whether exogenous silica grit in the atmosphere or opaline phytoliths
40 in plant matter. This is an important issue on two scales: macrowear, the progressive removal of
41 dental tissue over a significant portion of the tooth height, threatening the long-term survival of
42 **many herbivores and omnivores**; and microwear, the pattern of pit and scratch markings used to
43 infer dietary histories in fossil remains. One group of authors, employing precision nanoscratch
44 experiments on individual particulates, has argued that phytoliths are ineffective agents of wear
45 (5-7). Echoing an earlier study (8), they posit that while phytoliths are basically silicate in
46 composition, their hardness is significantly lower than that of exogenous silica particles and even
47 enamel, thus too soft to effect meaningful tooth wear. Another group challenges this assertion
48 by questioning that interpretation and the reliability of hardness tests on small-scale specimens
49 (9,10). Consequently, there **is need** for clarity in the roles of competing particulate types in the
50 broader discussion of tooth wear.

51 What appears to be **missing is** systematic macrowear evidence—can phytoliths cause
52 full-scale enamel loss under long-term abrasive conditions? In this report we present results of
53 macrowear tests on extracted human molar teeth in an artificial saliva medium, with and **without**
54 **phytoliths** or silica grit. **A** conventional pin-on-disk wear **test**, with the tooth cusp as pin on a flat
55 antagonist plate under conditions commensurate with normal biting **force, enables** us to separate
56 the mechanics of particle-on-enamel abrasion from the complex processes of real-life chewing.
57 We demonstrate that phytoliths can indeed **cause wear**, comparable to that of silica grit of similar
58 scale. Implications concerning the existing microwear dialog are considered.

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62 2. MATERIALS AND METHODS

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64 Impacted human wisdom teeth extracted from healthy adults (CICOM clinic, Badajoz, Spain) and free of
65 surface damage were cleaned and kept in aqueous solution. Ensuing wear experiments were run within
66 one week of **extraction**. Secondary cusps and roots were machined away from each tooth and the bases
67 ground flat. The prepared teeth were placed in 3 groups of 5 for subsequent wear testing in different
68 media.

69 Phytoliths were obtained by acid extraction from wheat stems and leaves (Supplementary
70 Information). The moderate temperature acid extraction used in the plant processing is not expected to
71 change the phytolith physical properties (11). Silica grit was obtained from a commercial source (A.
72 Anglada Suministros Ceramicos, Zaragoza, Spain). Both phytolith and silica **powders** were sieved to
73 remove particulates $> 80 \mu\text{m}$. Image analysis software (Image-Pro Plus 6.0, Media Cybernetics Inc.,
74 Rockville MD) was used to analyze size distributions of the two particle groups from photographic
75 images, before and after testing. Maximum longitudinal dimensions L and lateral dimensions D were
76 evaluated from some 120 particles/group. Each group of particles was dispersed by sonication for several
77 minutes at a concentration 0.5 vol% in a test medium of an artificial saliva (Lacer S.A., Barcelona, Spain)
78 immediately prior to testing.

79 Tests were conducted in a pin-on-disk wear machine (Tribometer THT, Anton Paar, Graz,
80 Austria) at room temperature. Each molar cusp was glued onto a pin holder mounted at a distance 3 mm
81 from the machine axis (Supplementary Information). The molar cusps were placed in contact with a
82 highly polished dental-zirconia disk and immersed in the saliva/saliva+particles medium. A load of 30 N
83 was applied to the specimen holder, and the disk rotated at 32 revs/min for 250 min, corresponding to a
84 sliding distance $\sim 150 \text{ m}$. The medium was replenished after each test run.

85 The molar cusp geometry was mapped by digital optical profilometry (Profilm, Filmetrics, San
86 Diego, CA) before and after wear testing (Supplementary Information). Measurements were made of
87 initial molar cusp radius R , wear height h and scar cross section area A . Wear volume was determined as
88 hA (Supplementary Information). Optical microscopy (Epiphot 300, Nikon, Tokyo, Japan) and scanning

89 electron microscopy (SEM S-3600, Hitachi, Tokyo, Japan) were used to examine wear tracks on the scar
90 surfaces.

91

92 3. RESULTS

93

94 SEM images of the silica grit and phytoliths are shown in Fig. 1, along with distributions of
95 particle dimensions from image analysis. Means and standard deviations were: longitudinal
96 dimension $L = 30 \pm 11 \mu\text{m}$ and aspect ratio $L/D = 1.5 \pm 0.5$ for silica; $L = 24 \pm 16 \mu\text{m}$ and L/D
97 $= 2.2 \pm 1.3$ for phytoliths. The phytoliths are a little smaller and somewhat more elongate,
98 flatter and sharper than the silica, but their longitudinal dimensions lie in the same broad range.
99 The particulates remained intact through the test duration (Supplementary Information), despite
100 having inflicted substantial wear on the enamel specimens.

101 Figure 2a shows profilometer traces of a typical molar cusp before and after wear testing,
102 from which cusp radius R , scar height h and area A could be digitally evaluated (Supplementary
103 Information). Representative wear volumes hA for the entire data set are plotted on the bar chart
104 in Fig. 2b, means and standard deviations 5 specimens/group. Student t-tests indicate that the
105 differences between tests in media with and without either silica or phytolith additives are
106 significant ($p < 0.05$), but that differences between the two particle types are not ($p > 0.05$)
107 (Supplementary Information). T-tests on the tabulated cusp radius data R indicate no significant
108 difference in effective tooth size between groups.

109 Figure 2c compares SEM images of wear facets on the molar pins for saliva (S), saliva
110 plus phytoliths (S+P) and saliva plus silica grit (S+S) media. Whereas the scar in image S shows
111 relatively fine tracks with some surface smearing, those in images S+P and S+S reveal μm -scale
112 gouges from individual particle microcontacts, indicative of wear in the severe abrasion region
113 (12).

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115

116 4. DISCUSSION

117

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

126 The question may be raised as to how demonstrative these data are of natural tooth wear
127 in mammals, especially in the knowledge that wear tests can be variable. Real-world mastication
128 is complicated by the convoluted nature of occlusal contact motion over prolonged time scales in
129 different species and by a wide range in food properties. Chewing machines can take us closer to
130 reality (13,14), but at some expense in analytical simplicity. The conditions of our basic wear
131 tests are not atypical of tooth function for human molars (15): normal load of 30 N per cusp,
132 occlusal facet diameter ~ 0.5 mm, simulated saliva medium. Each test corresponds to $150 \text{ m}/0.5$
133 $\text{mm} = 300,000$ passes (sliding distance/wear scar diameter), i.e. toward a lifetime of mastication
134 in humans. It might be argued that since our tests are run against a hard (zirconia) antagonist,
135 our observations represent a worst-case scenario. However, contact damage in brittle materials
136 is relatively insensitive to the antagonist material, provided the latter is at least as hard as the
137 specimen (16). The mode of material removal described in the current tests, most apparent in
138 Fig. 2c, indisputably falls into the category of 'severe abrasive macrowear' (12). One group of
139 researchers (5) in scratch tests with individual particles attached to a nanoindenter tip have
140 concluded that phytoliths cause only 'rubbing' marks on enamel, with material displacement
141 rather than removal. Their experiments lie in the domain of microwear, with evolutionary
142 implications in the interpretation of residual markings on fossil teeth. Based on instrumented

143 nanoindentation hardness measurements on individual particles (6,8), Lucas *et al.* conclude that
144 phytoliths are too soft to cause abrasive wear on enamel. Their quoted hardness ranges for
145 typical phytoliths are substantially lower than those for silica grit, and marginally lower than
146 those for enamel. Others (10) contest this conclusion, arguing that nanoindentation experiments
147 on such small particulates cannot be considered reliable, and that the hardness values quoted are
148 inconsistent with those from previous workers. In this context, it has been found from
149 indentation plasticity theory that particles with hardness as low as one third that of another body
150 may still scratch that second body (16), a contention supported in reciprocating sliding
151 microwear tests with soft metal spheres on enamel (17). Moreover, as acknowledged by Lucas
152 *et al.*, even displaced material becomes susceptible to removal in subsequent, multiple scratch
153 events. Recall that even tests in particle-free saliva, with their relatively fine surface scratches
154 (Fig. 2c), still produce measurable wear (Fig. 2b).

155 This is not to dispute the essential findings of Lucas *et al.*, but simply to point out that
156 their scratch experiments are conducted in a low-load 'subthreshold' region, where contact
157 deformation is exclusively plastic, whereas our current tests are conducted in a comparatively
158 high-load 'post-threshold' region, where microcracks may coalesce and facilitate the kind of
159 abrasive wear seen in Fig. 2c (18). The onset of microcracking may be aggravated by sharp
160 phytolith corners (19), and ensuing coalescence to be cumulative in multiple sliding contacts
161 (20). Extensive damage of this kind is most likely to prevail in prolonged high-load occlusal
162 mastication, as with some mammals who wear their teeth down to the gumline. Whether
163 endogenous phytoliths or exogenous grit dominate in the wear of mammalian dentition in nature
164 comes down to diet and environment—teasing apart these competing sources in relation to
165 microwear and macrowear has proved challenging. It is plausible that encounters with
166 postthreshold abrasive contacts of any complexion are sufficiently sparse as to allow plastic flow
167 to dominate microwear patterns in some animals, but at the same time sufficiently frequent to
168 exacerbate abrasive macrowear over extended lifetimes in others. Regardless, the evidence is

169 compelling that phytoliths are capable of producing tooth macrowear, if only in severe
170 conditions, comparable in degree to that of silica grit.

171

172 **Acknowledgements**

173

174 Extracted molars were provided by Dr Florencio Monje Gil (CICOM clinic). Funding was
175 provided by the Junta de Extremadura, Spain, FEDER/ERDF (grants IB16139 and GR18149)
176 and the Ministry of Science and Innovation (grant PID2019-105377RB-I00). Approval to
177 conduct this research was granted by the Bioethics Committee of the University of Extremadura.

178

179 **Figures**

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

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186 2. Evaluation of wear. (a) Profilometer trace of typical human molar cusp before and after
187 wear testing, showing height h and area A of scar. Vertical axis exaggerated. (b) Bar
188 chart plotting mean and standard deviation of wear volume parameter hA for each group
189 of 5 specimens in the 3 test media. (c) Wear scars on specimen tested in saliva medium
190 (S), saliva plus phytoliths (S+P) and saliva plus silica grit (S+S).

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