Received: 8 March 2020 | Accepted: 12 January 2021

DOI: 10.1111/eos.12771

European Journal of Oral Sciences

ORIGINAL ARTICLE

The effect of phytic acid on enzymatic degradation of dentin

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Funding information
The authors received no financial support for the research and/or authorship of this article.

Abstract

We evaluated the effect of phytic acid on matrix metalloproteinase (MMP)- or cysteine cathepsin (CC)-mediated dentin degradation. Demineralized dentin beams were divided into five groups (n = 12) and treated with 1%, 2%, or 3% phytic acid or with 37% phosphoric acid. Untreated demineralized beams served as controls. After incubation for 1 or 3 wk, dry mass loss was determined and aliquots of incubation media were analysed for cross-linked telopeptide of type I collagen (ICTP) fragments for MMP-mediated and c-terminal telopeptide of type I collagen (CTX) for cathepsin-k-mediated degradation. The direct effect of phytic acid was evaluated using MMP activity assay. Data were analysed using repeated-measures ANOVA. ICTP releases with 1% and 2% phytic acid treatment were statistically significantly lower than those following phosphoric acid treatment at 3 wk. The CTX release for phytic acid-treated beams at 3 wk was not significantly different from that of untreated control beams, but it was significantly lower than that of phosphoric acid-treated beams. Their MMP activities at 3 wk were not significantly different from those of the controls but they were significantly lower than those seen for phosphoric acid-treated beams. Compared to phosphoric acid, phytic acid treatment resulted in a reduced dentinal host-derived endogenous enzymatic activity and collagen degradation.

KEYWORDS

cathepsin, collagen, demineralized, MMPs, phytic acid

INTRODUCTION

Surface treatment of dental hard tissues is a prerequisite for the removal of the smear layer and for the facilitation of micromechanical retention of adhesive resins (1,2). Since the introduction of adhesive bonding, phosphoric acid treatment has been widely used as a surface treatment to demineralize and facilitate bonding to enamel and dentin (3,4). Although the infiltration of adhesive resins into acid-etched enamel is shown to be successful and very stable over time (5), resin infiltration into acid-etched dentin is prone to continuous degradation resulting in the compromised longevity of restorations (6,7). Ideally, the collagen network of the demineralized dentin must be completely resin-infiltrated and polymerized to provide a continuous collagen/resin network that can successfully anchor the

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restoration to dentin (2). However, suboptimal infiltration of resin monomers into the collagen network of acid-etched dentin (8) leaves unprotected collagen fibrils that are susceptible to host-derived enzymatic degradation (9,10). Matrix metalloproteinases (MMPs) and cysteine cathepsins (CC) are two classes of host-derived endogenous proteases that are abundant in the pulp-dentin complex and in caries-affected dentin (10–12). Acid etching of dentin with phosphoric acid has been shown to activate the proteolytic enzymes in dentin (13) and modulate the expression and activity of these enzymes in a concentration-dependent manner (14).

Several approaches have been suggested to slow down the enzymatic activity associated with the use of phosphoric acid on dentin; these approaches include antimicrobial agents (15,16), solvents (17,18), ethanol wet-bonding technique (19), and the use of cross-linking agents (20,21). Previous attempts have been made to evaluate other etching agents, such as maleic acid, citric acid (22,23), or ethylenediaminetetraacetic acid (24). Recently, an etching agent called phytic acid has been reported to enhance resin-dentin bond strength while being biocompatible to pulpal (25) and osteoblast cells (26). This highly negatively charged agent can remove the smear layer due to its ability to chelate with positively charged cations (27,28). The exact mechanism through which phytic acid enhances the bond strength is still not fully understood, but it is thought to cross-link the exposed collagen network (29,30). It is worth mentioning that phytic acid is the major storage form of phosphorus in plant seeds and bran (31), and that it is produced at low-cost from rice bran (32).

Until now, no studies have evaluated the effect of phytic acid on dentinal MMP and CC activities. Thus, the purposes of this study were to measure the MMP and CC activities of demineralized dentin matrices before and after exposure to various concentrations of phytic acid, and to evaluate the direct effect of phytic acid on endogenous dentinal MMPs and the mass of demineralized dentin. The null hypothesis was that exposure of demineralized dentin to phytic acid does not affect the activity of MMPs or CC or the mass of the demineralized dentin.

MATERIAL AND METHODS

Sixty extracted non-carious third molars (stored at 4°C in 0.9% NaCl containing 0.02% sodium azide to prevent microbial growth) were used within 1 month after extraction. The teeth used were extracted sound human molars collected from anonymous donors and, therefore, were exempt from ethical notification according to the Finnish law (Tissue act, section 20). All chemicals, unless otherwise stated, were purchased from Sigma-Aldrich.

Specimen preparation

The enamel and superficial dentin of each tooth were removed, and beams (1 x 2 x 6 mm) were sectioned from the mid-coronal dentin using a low-speed saw (Isomet; Buehler) under water-cooling. The beams were then demineralized in 0.5 M ethylenediaminetetraacetic acid (EDTA) for 2 wk at 4°C in a shaking bath, rinsed in distilled water at 4°C for 2 h, and dried in vacuum desiccators containing anhydrous silica beads for 72 h to assess their initial dry mass. After dry mass measurements, the beams were distributed to different groups so that the mean dry mass of each group was not statistically different. Beams were divided into five groups (n = 12 per group) and each group was treated with either 37% phosphoric acid or 1%, 2%, or 3% phytic acid (Wako Pure Chemical Industries) for 1 min. The control group consisted of untreated demineralized dentin beams. After treatment, each beam was blot-dried and incubated in 2 mL individually-labelled screw-top polypropylene tubes (HS10060; Sigma-Aldrich) with 1 ml artificial saliva with a pH of 7.2 and containing 5 mM HEPES, 2.5 mM $CaCl_2.H_2O$, 0.02 mM $ZnCl_2$, and 0.3 mM NaN_3 (33) at 37°C for 1- and 3-wk incubation in a shaking-water-bath (60 cycles/min).

Loss of dry mass

The enzymatic degradation of dentin matrix, due to the hydrolytic and solubilization process of total protease activity, was indirectly measured by the loss of demineralized dry dentin mass. Initial demineralized dentin beams, rinsed in 1 ml distilled water at 4°C for 24 h, were transferred to individually-labelled 96-well plates and placed in desiccators containing dry silica beads for 72 h. Samples were then weighed individually with an analytical balance (XP6 Microbalance; Mettler Toledo). After dry mass measurement, beams were rehydrated for 2 h in distilled water at 4°C and incubated in 0.5 ml artificial saliva, as described above, for 1 and 3 wk at 37°C in a shaking bath. After each incubation period, beams were rinsed in distilled water at 4°C for 24 h and dehydrated for 72 h, and dry mass loss was re-assessed. The loss of dry mass was calculated as the percentage change of dry mass loss referring to the initial dry mass recorded for each beam.

Generic MMP assay

To evaluate whether phytic acid can alter the activity of dentin MMPs, a generic colorimetric MMP assay (Sensolyte Generic Colorimetric MMP assay; AnaSpec) was used in this study. After demineralization, rehydrated beams (2 h at 4°C) were

incubated in 150 µl of chromogenic substrate and assay buffer in a 96-well plate for 60 min at 25°C. After incubation, the beams were removed and baseline MMP activity of each beam was measured at 412 nm using a colorimetric spectrometer (Synergy HT; BioTek Instruments). After baseline measurements, beams were rinsed free of MMP assay substrate and distributed into five groups (n = 12 per group) as described above. The beams were then dipped in 300 µL of 37% phosphoric acid, 1%, 2%, or 3% phytic acid or water (control) solution for 1 min, rinsed for 30 s, blot-dried, and incubated in fresh chromogenic thiopeptide substrate and assay buffer provided by the manufacturer (Sensolyte Generic MMP Colorimetric assay; AnaSpec) and re-assessed, as described above. The total MMP activity of each treatment group was expressed as a percentage of the baseline measurement for each individual beam to determine the relative inhibition or activation (34). Quadruplicate analyses were performed for each incubation period and for each concentration of phytic acid and phosphoric acid.

Solubilized telopeptides of collagen

Type I collagen degradation, mediated by MMP and CC proteases, results in the release of specific c-telopeptide fragments (16,35,36). Cross-linked carboxyterminal telopeptide of type I collagen (ICTP) is solubilized by MMP activity, whereas cathepsin-k, among the CCs, degrades c-terminal telopeptide of type I collagen (CTX) (16,35). To analyse the amount of solubilized fragment after each incubation period, commercial ELISA kits for ICTP (UniQEIA; Orion Diagnostica) and for CTX (Crosslaps ELISA; Immuno Diagnostics System) were used. After each incubation period (1 and 3 wk), 0.5 ml aliquots of incubation media were retrieved from each tube and 20–25 µl of the incubation medium were used to measure solubilized ICTP and CTX fragments. The measurement was performed using a spectrometer (Synergy HT; BioTek) at 450 nm absorbance and the amount of ICTP or CTX release was calculated according to the standard curve using the standards with known concentrations provided in the kits.

Statistical analyses

The percent loss of dry mass, the amount of released ICTP and CTX, and the percent of MMP activity in each incubation time were estimated using mean values and standard deviation and were checked for significant deviation from normality (Kolmogorov-Smirnov test) and homoscedasticity (Modified Levene's test). When the normality and equality variance assumptions of the data were valid, they were analysed using a repeated-measures analysis of variance (ANOVA). When the data could not be transformed into a normal distribution, the data were analysed with Kruskal-Wallis test, followed by Dunn's multiple comparison test. Post hoc multiple comparisons were performed with the Tukey test using SPSS (version 21; IBM). Statistical significance was pre-set at α=0.05.

RESULTS

Dry mass loss

Loss of dry mass from demineralized dentin beams treated with phosphoric acid or various concentrations of phytic acid is depicted in Figure 1. The control group with no acid treatment showed around 5%–6% decrease in dry mass, while phosphoric acid-treated beams showed a higher (15%) dry

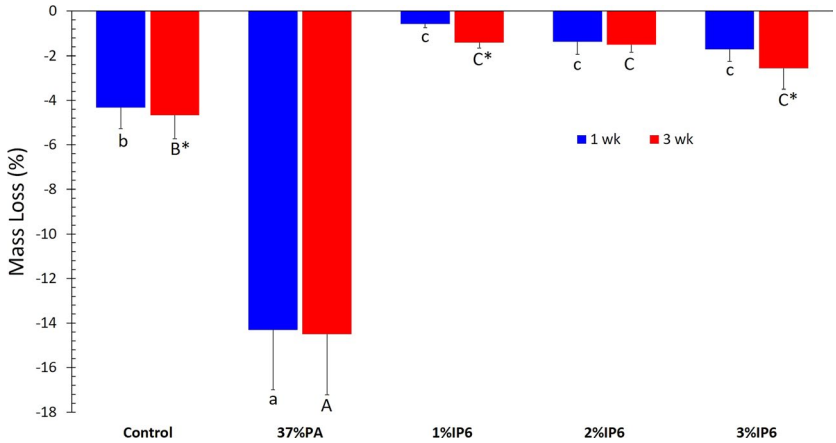

FIGURE 1 Loss of dry mass from demineralized dentin beams treated with either phosphoric acid (PA) or 1%, 2%, or 3% phytic acid (IP6) after 1 wk or 3 wk of incubation were estimated using mean values and standard deviation. Groups after 1 wk of incubation time with the same lower-case letter designations are not statistically significant ($p > 0.05$). Groups after 3 wk of incubation time with the same upper-case letter designations are not statistically significant ($p > 0.05$). A statistical difference within the same group after 1 and 3 wk of incubation is represented by an asterisk (*). IP6 resulted in significantly lower mass losses when compared to the control and PA at both incubation times

mass loss. The dry mass losses in 1%, 2%, and 3% phytic acid-treated were between 1%–3%, all of which were significantly lower than those seen for phosphoric acid-treated beams ($p < 0.0001$). Interestingly, the dry mass losses of 1%, 2%, and 3% phytic acid-treated beams were statistically significantly lower than the control ($p < 0.0001$). The dry mass loss after 3 wk of incubation was significantly higher than that after 1 wk of incubation for the control ($p = 0.017$), 1% phytic acid ($p = 0.002$), and 3% phytic acid ($p = 0.024$); however, the difference was not significant for 2% phytic acid ($p = 0.35$) and phosphoric acid ($p = 0.51$).

Generic MMP assay

When EDTA-demineralized dentin beams were used as a source of MMP activity, the baseline activity of all beams was not significant among different groups ($p = 0.92$) (Figure S1). The total generic MMP activity (in percentage) after treatment with respective solutions is presented in Figure 2. There was no statistically significant difference among different concentrations of phytic acid and control. For incubation times, only phosphoric acid showed a significant difference in the percent of total MMP activity over time, in which immediate treatment produced the highest MMP activity, followed by 3 wk and then 1 wk of incubation ($p < 0.05$). However, at 1 wk of incubation time all beams had an MMP activity that was not statistically significantly different from the other treatments ($p = 0.6$). It is worth mentioning that MMP activities of phytic acid beams after 3 wk of incubation were not significantly different from control ($p > 0.05$), but they were significantly lower than that seen for phosphoric acid ($p = 0.001$).

Cross-linked telopeptide of type I collagen release

The quantities of ICTP telopeptides released after incubation of the demineralized dentin beams for 1 and 3 wk are presented in Figure 3. The control, as well as 1% and 2% phytic acid-treated specimens, had significantly lower ICTP releases than phosphoric acid-treated specimens after 3 wk of incubation ($p < 0.05$), while 3% phytic acid was not significantly different when compared to phosphoric acid ($p = 0.78$). Interestingly, the ICTP released with 3% phytic acid after 1 wk of incubation was higher than the amount of release obtained with phosphoric acid at the same incubation time, even though there was no significant difference ($p = 0.246$). Phosphoric acid-treated beams showed significantly higher release at 3 wk compared with 1 wk ($p = 0.005$). A tendency for decreased ICPT release over time was observed in phytic acid-treated beams; however, this decrease did not reach the level of significance with 3% phytic acid ($p = 0.051$), while it was significant for 1% phytic acid ($p = 0.004$) and 2% phytic acid ($p = 0.038$).

C-terminal telopeptide of type I collagen release

The quantities of CTX released after incubation of the demineralized dentin beams for 1 and 3 wk are presented in Figure 4. All phytic acid-treated beams had significantly lower CTX releases when compared to phosphoric acid at both incubation times ($p < 0.05$). The released CTX of all phytic treated beams after 1 wk of incubation time were significantly higher than the control ($p < 0.05$). While the released CTX of all phytic acid-treated beams after 3 wk of incubation was not

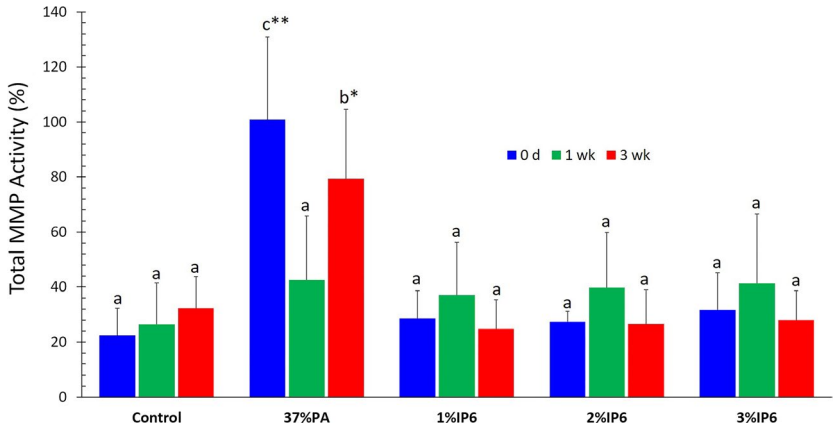

FIGURE 2 The total matrix metalloproteinases (MMP) activity of demineralized dentin treated with either phosphoric acid (PA) or 1%, 2%, or 3% phytic acid (IP6) after 1 wk or 3 wk of incubation were estimated using mean values and standard deviation. The mean of total MMP activity is shown as the % change compared to baseline level. Groups with the same lower-case letter designations are not statistically significant ($p > 0.05$). After 3 wk of incubation, IP6 resulted in MMP activity that is lower compared to PA but is similar to that of the control. For incubation times within the same group, only PA showed a significant difference in % total MMP activity over time, in which immediate treatment produced the highest MMP activity, followed by 3 and 1 wk of incubation, respectively, and the differences are represented by asterisk (*)

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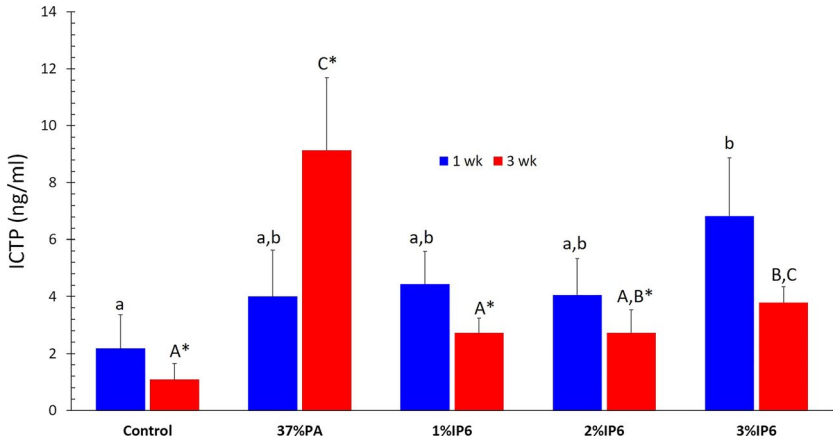

FIGURE 3 The rate of crosslinked carboxyterminal telopeptide of type I collagen (ICTP) release from dentin beams treated with either phosphoric acid (PA) or with 1%, 2%, or 3% phytic acid (IP6) after 1 wk or 3 wk of incubation were estimated using mean values and standard deviation. Groups of 1 wk of incubation time with the same lower-case letter designations are not statistically significant ($p > 0.05$). Groups of 3 wk of incubation time with the same upper-case letter designations are not statistically significant ($p > 0.05$). A statistical difference within the same group after 1 and 3 wk of incubation is represented by an asterisk (*). After 3 wk of incubation, 1% and 2% IP6 resulted in lower ICTP release compared to PA but they were similar to that of the control. All groups showed either lower or similar ICTP release after 3 wk of incubation when compared to 1 wk, except that PA which showed higher ICTP release after 3 wk when compared to 1 wk of incubation

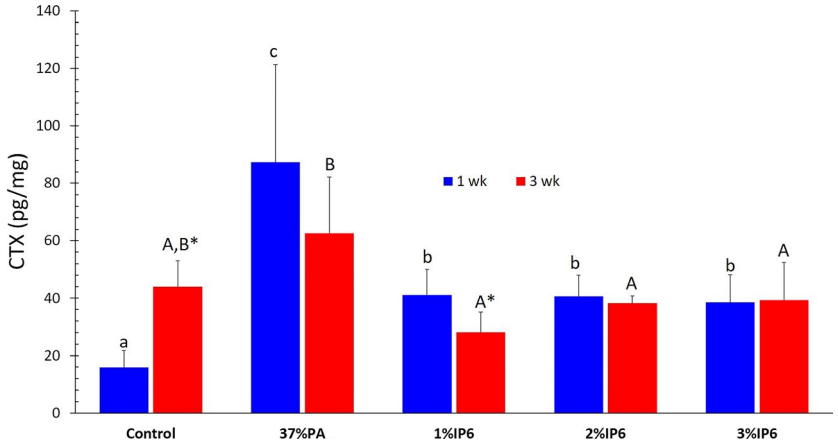

FIGURE 4 The rate of c-terminal telopepetide of type I collagen (CTX) release from dentin beams treated with either phosphoric acid (PA) or with 1%, 2%, or 3% phytic acid (IP6) after 1 wk or 3 wk of incubation were estimated using mean values and standard deviation. Groups of 1 wk of incubation time with the same lower-case letter designations are not statistically significant ($p > 0.05$). Groups of 3 wk of incubation time with the same upper-case letter designations are not statistically significant ($p > 0.05$). A statistical difference within the same group after 1 and 3 wk of incubation is represented by an asterisk (*). At both incubation times, IP6 resulted in lower CTX release when compared to PA. IP6 showed a similar CTX release to the control after 3 wk of incubation

significantly different from control ($p > 0.05$), the released CTX in 1% phytic acid was significantly lower after 3 wk when compared to 1 wk of incubation ($p = 0.001$).

DISCUSSION

In this study, dentinal collagen matrix degradation was studied by evaluating the release of ICTP and CTX fragments from degrading collagen fibrils, and the amount of mass loss and MMP activity. The results of the present study have shown that phytic acid treatment results in less mass loss in demineralized dentin than seen for untreated control beams and for phosphoric acid-treated beams. Treatment with 1% and 2% phytic acid resulted in less ICTP release from dentin after 3 wk of incubation than did treatment with phosphoric acid. The phytic acid-induced CTX release was comparable to that seen in untreated beams at 3 wk, while it resulted in a significantly lower CTX release than did phosphoric acid treatment. These results require rejection of the null hypotheses.

Demineralized dentin beams treated with 1% or 2% phytic acid showed the least dry mass loss, which was less than half the loss observed in the control group and 7 times lower than the loss obtained in dentin beams treated with phosphoric acid. Dry mass loss is an indirect measure of solubilization of collagen from the dentin matrix (20). Loss of dry mass over time

indicates the solubilization of collagen matrix by activated endogenous enzymes. In similar studies, dentin cross-linkers have been reported to inhibit or completely stop mass loss after 1 wk of incubation (37). We speculate that a cross-linking action of phytic acid is obtained on dentin collagen based on previous reports on its cross-linking abilities to protein and chitosan (38,39). This speculated cross-linking action might positively affect the mechanical strength of the collagen. Increased stiffness of dentinal collagen might lead to less susceptibility of the collagen to degradation. The interaction between phytic acid and protein is reported to be in the form of direct electrostatic interaction (40). The cross-linking effect of phytic acid occurs through the binding of anions of phytic acid with the cations of proteins (38). It is known that the dentinal collagen would have a positive net charge upon exposure to acidic solutions (41,42) and, thus, we speculate that the aforementioned interaction occurs between phytic acid and demineralized dentinal collagen. However, the interaction of phytic acid with protein might also take different forms; the binary interaction described above occurs mainly at a low pH, below the isoelectric point of the protein where the net charge of the protein is positive. This type of complex is described as insoluble and may dissolve only below a pH of 3.5. Tertiary protein-cation-phytic acid complexes occur at a higher pH, above the isoelectric point of the protein where the net charge is negative (43,44). A new and less studied theory of interaction with protein is the ability of phytic acid to act as a Hofmeister anion (enhanced by its six anionic groups), resulting in a kosmotropic effect that stabilizes proteins by interacting with water in the surrounding medium (44). Overall, these interactions may induce changes in the protein structure that result in decreased enzymatic activity, protein solubility, and proteolytic digestibility (43). These mechanisms of action might explain the lower mass losses in the phytic acid-treated beams when compared to the untreated control group. However, the formation of insoluble phytic acid-collagen complexes that are difficult to rinse off might have also contributed to this finding.

Cross-linked telopeptide of type I collagen telopeptide release peaked in the control and phytic acid-treated beams after 1 wk of incubation, then significantly declined after 3 wk of incubation in the untreated control beams and in 1% and 2% phytic acid-treated beams; however, the release increased for phosphoric acid-treated beams. Release of ICTP is used to evaluate the inhibitory effect of agents on dentinal MMPs (45). Despite the results obtained in the present study, we do not think that phytic acid has a direct interaction with MMP. In this study, phytic acid-treated beams showed similar total MMP activity after 1 and 3 wk of incubation when compared to the untreated control beams. There is a lack of understanding of the possible interaction between phytic acid and MMP. A recent report revealed that phytic acid fails to dock with MMP-2 or MMP-9 due to its poor binding ability (46). The impact of phytic acid on MMPs in living tissue is said to be indirect and occur through the downregulation of genes expressing certain proteinases (47). In this study, the CTX telopeptide release increased after 3 wk of incubation time for the untreated control group, while it declined or remained stable for the other groups. Release of CTX from demineralized dentin is an indirect measure of cathepsin activity. One of the postulated mechanisms of cathepsin inhibition is the electrostatic binding between positively charged cross-linking agents and the cathepsin active site (48). This mechanism of interaction is not thought to be the reason for the results obtained in our study, so further studies are needed to explore the exact mechanism involved.

A variety of MMPs inhibitors have been studied in dentistry, and the research is still ongoing to further develop and find alternative agents that are both effective and non-toxic (49,50). Calcium and zinc are needed to maintain the structure and active site of MMPs (51); thus, chelation to zinc and calcium is suggested as one of the mechanisms by which to lower the activity of dentinal MMPs, and agents that function through this mechanism are known as first-generation MMP inhibitors (52). Phytic acid has an immense ability to chelate with calcium and zinc. Several factors affect the solubility of calcium-phytic acid complexes, such as pH and ratio of the cation to phytic acid. At low pH, these complexes are soluble, while at higher pH (> 4), insoluble complexes form (40,53,54). Zinc-phytic acid complexes are also reported to be stable and insoluble (55). At high calcium levels, phytic acid can form calcium-zinc-phytic acid complexes that are even less soluble than phytic acid complexes formed in either ion alone (56). Besides these interactions, phytic acid can also interact with several types of enzymes (such as proteinases), and most of these interactions lead to a reduction in the proteolytic activity of the enzyme (43). Phytic acid interaction with amino groups was also reported to reduce tissue degradability by blocking the collagenase action through obstructing and protecting the cleavage site (57); the complexes formed due to these interactions are more difficult to degrade enzymatically and, thus, higher concentrations of enzymes are needed to degrade these complexes (44,58).

In conclusion, phytic acid treatment of dentin resulted in less total dentinal collagen degradation than seen with phosphoric acid treatment. The exact mechanism by which phytic acid exerts this effect is not fully understood, but the most plausible explanations are through its interaction or cross-linking effect on collagen, chelation with calcium and/or zinc, or direct interaction with the degrading enzymes. Further studies are needed to determine the long-term effectiveness of phytic acid treatment and to detect the exact mechanisms by which it decreases dentinal collagen degradation.

ACKNOWLEDGEMENTS

Dr. Diletta Forgione (first author) and Dr. Mohannad Nassar (second author) contributed equally to this work.

European Journal of Oral Sciences NOF Scandinavian Division IADR

CONFLICT OF INTERESTS

The authors declare no potential conflicts of interest with respect to the authorship and/or publication of this article.

AUTHOR CONTRIBUTION

Conceptualization: Mohannad Nassar, Diletta Forgione; **Methodology:** Diletta Forgione, Roda Seseogullari-Dirihan, Arzu Tezvergil-Mutluay; **Software:** Roda Seseogullari-Dirihan, Suppason Thitthaweerat, Mohannad Nassar; **Validation:** Mohannad Nassar, Arzu Tezvergil-Mutluay; **Formal analysis:** Roda Seseogullari-Dirihan, Suppason Thitthaweerat, Mohannad Nassar; **Investigation:** Mohannad Nassar, Diletta Forgione; **Resources:** Arzu Tezvergil-Mutluay, Diletta Forgione; **Data Curation:** Diletta Forgione, Roda Seseogullari-Dirihan, Mohannad Nassar; **Writing - Original Draft:** Mohannad Nassar; **Writing - Review & Editing:** Mohannad Nassar, Arzu Tezvergil-Mutluay; **Visualization:** Mohannad Nassar, Diletta Forgione; **Supervision:** Mohannad Nassar, Arzu Tezvergil-Mutluay; **Project administration:** Arzu Tezvergil-Mutluay, Mohannad Nassar.

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REFERENCES

1. Van Meerbeek B, Yoshihara K, Yoshida Y, Mine A, De Munck J, Van Landuyt KL. State of the art of self-etch adhesives. Dent Mater. 2011;27:17–28.
2. Pashley DH, Tay FR, Breschi L, Tjäderhane L, Carvalho RM, Carrilho M, et al. State of the art etch-and-rinse adhesives. Dent Mater. 2011;27:1–16.
3. Lopes GC, Thys DG, Klaus P, Oliveira GM, Widmer N. Enamel acid etching: a review. Compend Contin Educ Dent. 2007;28:18–24; quiz 25:42.
4. Söderholm KJ. Dental adhesives …. how it all started and later evolved. J Adhes Dent. 2007;9(Suppl 2):227–30.
5. Gurel G, Morimoto S, Calamita MA, Coachman C, Sesma N. Clinical performance of porcelain laminate veneers: outcomes of the aesthetic pre-evaluative temporary (APT) technique. Int J Periodontics Restorative Dent. 2012;32:625–35.
6. De Munck J, Van Meerbeek B, Yoshida Y, Inoue S, Vargas M, Suzuki K, et al. Four-year water degradation of total-etch adhesives bonded to dentin. J Dent Res. 2003;82:136–40.
7. Hashimoto M, Ohno H, Kaga M, Endo K, Sano H, Oguchi H. In vivo degradation of resin–dentin bonds in humans over 1 to 3 years. J Dent Res. 2000;79:1385–91.
8. Sauro S, Pashley DH, Mannocci F, Tay FR, Pilecki P, Sheriff M, et al. Micropermeability of current self-etching and etch-and-rinse adhesives bonded to deep dentin: a comparison study using a double-staining/confocal microscope technique. Eur J Oral Sci. 2008;116:184–93.
9. Pashley DH, Tay FR, Yiu C, Hashimoto M, Breschi L, Carvalho RM, et al. Collagen degradation by host-derived enzymes during aging. J Dent Res. 2004;83:216–21.
10. Tjaderhane L, Nascimento FD, Breschi L, Mazzoni A, Tersariol ILS, Geraldeli S, et al. Optimizing dentin bond durability: control of collagen degradation by matrix metalloproteinases and cysteine cathepsins. Dent Mater. 2013;29:116–35.
11. Mazzoni A, Nascimento FD, Carrilho M, Tersariol I, Papa V, Tjaderhane L, et al. Matrix metalloproteinase (MMP) activity in the hybrid layers detected with in situ zymography. J Dent Res. 2012;91:467–72.
12. Vidal CM, Tjäderhane L, Scaffa PM, Tersariol IL, Pashley D, Nader HB, et al. Abundance of MMPs and cysteine cathepsins in caries-affected dentin. J Dent Res. 2014;93:269–74.
13. Tezvergil-Mutluay A, Mutluay M, Seseogullari-Dirihan R, Agee KA, Key WO, Scheffel DL, et al. Effect of phosphoric acid on the degradation of human dentin matrix. J Dent Res. 2013;92:87–91.
14. Devito-Moraes AG, Francci C, Vidal CM, Scaffa PM, Nesadal D, Yamasaki LC, et al. Phosphoric acid concentration affects dentinal MMPs activity. J Dent. 2016;53:30–7.
15. Hebling J, Pashley DH, Tjäderhane L, Tay FR. Chlorhexidine arrests subclinical degradation of dentin hybrid layers in vivo. J Dent Res. 2005;84:741–6.
16. Tezvergil-Mutluay A, Agee K, Mazzoni A, Carvalho RM, Carrilho M, et al. Can quaternary ammonium methacrylates inhibit matrix MMPs and cathepsins. Dent Mater. 2015;31:e25–32.
17. Tezvergil-Mutluay A, Agee KA, Hoshika T, Uchiyama T, Tjäderhane L, Breschi L, et al. Inhibition of MMPs by alcohols. Dent Mater. 2011;27:926–33.
18. Stape THS, Seseogullari-Dirihan R, Tjäderhane L, Abuna GL, Martins LRM, Tezvergil-Mutluay A. A novel dry-bonding approach to reduce collagen degradation and optimize resin-dentin interfaces. Sci Rep. 2018;8:16890.
19. Pashley DH, Tay FR, Carvalho RM, Rueggeberg FA, Agee KA, Carrilho M, et al. From dry bonding to water-wet bonding to ethanol-wet bonding. A review of the interactions between dentin matrix and solvated resins using a macromodel of the hybrid layer. Am J Dent. 2007;20:7–20.
20. Tezvergil-Mutluay A, Mutluay MM, Agee KA, Seseogullari-Dirihan R, Hoshika T, Cadenaro M, et al. Carbodiimide cross-linking inactivates soluble and matrix-bound MMPs, in vitro. J Dent Res. 2012;91:192–6.
21. Macedo GV, Yamauchi M, Bedran-Russo AK. Effects of chemical cross-linkers on caries-affected dentin bonding. J Dent Res. 2009;88:1096–100.
22. Breschi L, Gobbi P, Mazzotti G, Falconi M, Ellis TH, Stangel I. High resolution SEM evaluation of dentin etched with maleic acid and citric acid. Dent Mater. 2002;18:26–35.
23. Trevelin LT, Villanueva J, Zamperini CA, Mathew MT, Matos AB, Bedran-Russo AK. Investigation of five α-hydroxy acids for enamel and dentin etching: demineralization depth, resin adhesion and dentin enzymatic activity. Dent Mater. 2019;35:900–8.
24. Sauro S, Toledano M, Aguilera FS, Mannocci F, Pashley DH, Tay FR, et al. Resin-dentin bonds to EDTA-treated vs. acid-etched dentin using ethanol wet-bonding. Dent Mater. 2010;26:368–79.
25. Nassar M, Hiraishi N, Islam MS, Aizawa M, Tamura Y, Otsuki M, et al. Effect of phytic acid used as etchant on bond strength, smear layer, and pulpal cells. Eur J Oral Sci. 2013;121:482–7.

26. Nassar M, Hiraishi N, Tamura Y, Otsuki M, Aoki K, Tagami J. Phytic acid: an alternative root canal chelating agent. J Endod. 2015;41:242–7.
27. Barrientos LG, Murthy PPN. Conformational studies of myo-inositol phosphates. Carbohydr Res. 1996;296:39–54.
28. Cowieson AJ, Acamovic T, Bedford MR. Phytic acid and phytase: implications for protein utilization by poultry. Poult Sci. 2006;85:878–85.
29. Kong K, Hiraishi N, Nassar M, Otsuki M, Yiu CKY, Tagami J. Effect of phytic acid etchant on resin-dentin bonding: monomer penetration and stability of dentin collagen. J Prosthodont Res. 2017;61:251–8.
30. Kong K, Islam MS, Nassar M, Hiraishi N, Otsuki M, Yiu CKY, et al. Effect of phytic acid etchant on the structural stability of demineralized dentine and dentine bonding. J Mech Behav Biomed Mater. 2015;48:145–52.
31. Kuwano M, Mimura T, Takaiwa F, Yoshida KT. Generation of stable 'low phytic acid' transgenic rice through antisense repression of the 1D-myo-inositol 3-phosphate synthase gene (RINO1) using the 18-kDa oleosin promoter. Plant Biotechnol J. 2009;7:96–105.
32. Graf E. Applications of phytic acid. J Am Chem Soc. 1983;60:1861–7.
33. Tezvergil-Mutluay A, Agee KA, Hoshika T, Carrilho M, Breschi L, Tjäderhane L, et al. The Requirement of zinc and calcium ions for functional MMP activity in demineralized dentin matrices. Dent Mater. 2010;26:1059–67.
34. Ozcan S, Seseogullari-Dirihan R, Uctasli M, Tay FR, Pashley DH, Tezvergil-Mutluay A. Effect of polyacrylic acid on dentin protease activities. Dent Mater. 2015;31:901–6.
35. Garnero P, Borel O, Byrjalsen I, Ferreras M, Drake FH, Mcqueney MS, et al. The collagenolytic activity of cathepsin K is unique among mammalian proteases. J Biol Chem. 1998;273:32347–52.
36. Garnero P, Ferras M, Karsdal MA, Nicamhlaoibh R, Risteli J, Borel O, et al. The type I collagen fragments ICTP and CTX reveal distinct enzymatic fragments of bone collagen degradation. J Bone Min Res. 2003;18:859–67.
37. Scheffel DL, Hebling J, Scheffel RH, Agee KA, Cadenaro M, Turco G, et al. Stabilization of dentin matrix after cross-linking treatments, in vitro. Dent Mater. 2014;30:227–33.
38. Ravichandran R, Seitz V, Reddy Venugopal J, Sridhar R, Sundarrajan S, Mukherjee S, et al. Mimicking native extracellular matrix with phytic acid-crosslinked protein nanofibers for cardiac tissue engineering. Macromol Biosci. 2013;13:366–75.
39. Lee H, Jeong C, Ghafoor K, Cho S, Park J. Oral delivery of insulin using chitosan capsules cross-linked with phytic acid. Biomed Mater Eng. 2011;21:25–36.
40. Cheryan M. Phytic acid interactions in food systems. Crit Rev Food Sci Nutr. 1980;13:297–335.
41. Nezu T, Winnik FM. Interaction of water-soluble collagen with poly(acrylic acid). Biomaterials. 2000;21:415–9.
42. Zhang J, Senger B, Vautier D, Picart C, Schaaf P, Voegel JC, et al. Natural polyelectrolyte films based on layer by layer deposition of collagen and hyaluronic acid. Biomaterials. 2005;26:3353–61.
43. Greiner R, Konietzny U, Jany KD. Phytate-an undesirable constituent of plant-based foods? J für Ernährungsmedizin. 2006;8:18–28.
44. Selle PH, Cowieson AJ, Cowieson NP, Ravindran V. Protein-phytate interactions in pig and poultry nutrition: a reappraisal. Nutr Res Rev. 2012;25:1–17.
45. Tezvergil-Mutluay A, Agee KA, Uchiyama T, Imazato S, Mutluay MM, Cadenaro M, et al. The inhibitory effects of quaternary ammonium methacrylates on soluble and matrix-bound MMPs. J Dent Res. 2011;90:535–40.
46. Narayanaswamy R, Wai LK, Esa NM. Molecular docking analysis of phytic acid and 4-hydroxyisoleucine as cyclooxygenase-2, microsomal prostaglandin E synthase-2, tyrosinase, human neutrophil elastase, matrix metalloproteinase-2 and -9, Xanthine oxidase, squalene synthase, nitric oxide synthase, human aldose reductase, and lipoxygenase inhibitors. Pharmacogn Mag. 2017;13:S512–8.
47. Kapral M, Wawszczyk J, Jurzak M, Hollek A, Węglarz L. The effect of inositol hexaphosphate on the expression of selected metalloproteinases and their tissue inhibitors in IL-1β-stimulated colon cancer cells. Int J Colorectal Dis. 2012;27:1419–28.
48. Umer D, Yiu CK, Burrow MF, Niu LN, Tay FR. Effect of a novel quaternary ammonium silane on dentin protease activities. J Dent. 2017;58:19–27.
49. Nassar M, Hiraishi N, Shimokawa H, Tamura Y, Otsuki M, Kasugai S, et al. The inhibition effect of non-protein thiols on dentinal matrix metalloproteinase activity and HEMA cytotoxicity. J Dent. 2014;42:312–8.
50. Boelen GJ, Boute L, D'Hoop J, Ezeldeen M, Lambrichts I, Opdenakker G. Matrix metalloproteinases and inhibitors in dentistry. Clin Oral Investig. 2019;23:2823–35.
51. Visse R, Nagase H. Matrix metalloproteinases and tissue inhibitors of metalloproteinases: structure, function, and biochemistry. Circ Res. 2003;92:827–39.
52. Toledano M, Yamauti M, Osorio E, Osorio R. Zinc-inhibited MMP-mediated collagen degradation after different dentine demineralization procedures. Caries Res. 2012;46:201–7.
53. Grynspan F, Cheryan M. Calcium phytate: effect of pH and molar ratio on in vitro solubility. J Am Oil Chem Soc. 1983;60:1761–4.
54. Crea F, Crea P, De Robertis A. Speciation of phytate ion in aqueous solution. Characterisation of Ca-phytate sparingly soluble species. Chem Spec Bioavail. 2004;16:53–9.
55. Oatway L, Vasanthan T, Helm JH. Phytic acid. Food Rev Int. 2001;17:419–31.
56. Fordyce EJ, Forbes RM, Robbins KR, Erdman JW. Phytate X calcium/zinc molar ratios: are they predictive of zinc bioavailability? J Food Sci. 1987;52:440–4.
57. Wang X, Wen K, Yang X, Li L, Yu X. Biocompatibility and anticalcification of a biological artery immobilized with naturally-occurring phytic acid as the crosslinking agent. J Mater Chem B. 2017;5:8115–24.
58. Ravindran V, Bryden WL, Kornegay ET. Phytates: occurrence, bioavailability and implications in poultry nutrition. Poult Avian Biol Rev. 1995;6:125–43.

SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section.

How to cite this article: Forgione D, Nassar M, Seseogullari-Dirihan R, Thitthaweerat S, Tezvergil-Mutluay A. The effect of phytic acid on enzymatic degradation of dentin. *Eur J Oral Sci*. 2021;129:e12771. https://doi.org/10.1111/eos.12771