Original Paper

Caries Research

Caries Res 2001;35:211–215

Received: June 23, 2000
Accepted after revision: January 25, 2001

Effect of Time on the Remineralisation of Enamel by Synthetic Saliva after Citric Acid Erosion

M. Eisenburger[a], M. Addy[b], J.A. Hughes[b], R.P. Shellis[b]

[a]Department of Prosthetic Dentistry, Medical University of Hannover, Germany;
[b]Division of Restorative Dentistry, Dental School, Bristol, UK

Key Words
Artificial saliva · Citric acid · Dental erosion · Enamel · Remineralisation · Ultrasonication

Abstract
The aim of this in vitro study was to determine the influence of remineralisation time on rehardening of surface-softened enamel after citric acid erosion. Seven groups of 13 samples of human enamel were eroded in 0.3% citric acid at pH 3.2 for 2 h followed by profilometric measurements. Individual groups of specimens were placed in artificial saliva for 1, 2, 4, 6, 9 or 24 h. A control group was placed in isotonic saline for 24 h. After new profilometric measurements samples were ultrasonicated stepwise up to 480 s with profilometric measurements performed at 5, 30, 120, 240 and 480 s to measure the depth of surface softening. The control group had a softened surface layer of mean thickness 2.9 μm. Mineral deposition was seen at all remineralisation times by scanning electron microscopy. Exposure to artificial saliva for 1, 2 or 4 h produced a partial rehardening of the softened enamel; the additional surface losses produced by ultrasonication were lower and time delayed compared to the control group. Specimens remineralised for 6, 9 and 24 h showed little evidence of surface loss after ultrasonication. The data suggest that a complete rehardening of the softened enamel in vitro is reached after a remineralisation time of 6 h. These data are of clinical relevance to tooth wear. However, there is a need for studies in situ of enamel demineralisation and remineralisation.

Erosion by acidic beverages causes an inevitable loss of tooth structure. However, the surface of the remaining tissue is partly demineralised and thereby softened. The damage to this layer can be reduced by remineralisation. Remineralisation or rehardening of enamel has been studied, largely in vitro, and as they relate to dental erosion and caries. Various methods have been used to assess the remineralisation process in demineralised enamel. Direct techniques measure the mineral intake using microradiography [Arends and Gelhard, 1983; González-Cabezas et al., 1998; Iijima et al., 1999] or chemical analysis [Arends and ten Cate, 1981]. Indirect methods determine other physical characteristics of the enamel which change with mineral content, e.g. microhardness measurement [Arends and Gelhard, 1983; Klimek and Hellwig, 1989; Collys et al., 1993], or profilometry [Attin et al., 1999, 2000; Jaeggi and Lussi, 1999; Eisenburger et al., 2000, 2001]. The mechanical methods have the advantage that they measure directly the physical stability of the rehardened enamel. In a previous study, the stabilisation of surface-softened enamel by 24-hour remineralisation in artificial saliva was proven by demonstration of a return to a normal level of resistance to ultrasonication [Eisenburger et al., 2001]. However, a 24-

KARGER

Fax +41 61 306 12 34
E-Mail karger@karger.ch
www.karger.com

0008–6568/01/0353– 0211 $17.50/0

Accessible online at:
www.karger.com/journals/cre

Dr. Michael Eisenburger
Medical University of Hannover, Department of Prosthetic Dentistry
Carl-Neuberg-Strasse 1, D–30625 Hannover (Germany)
Tel. + 49 511 532 4769, Fax + 49 511 532 4790
E-Mail M.Eisenburger@gmx.de

hour period has little clinical relevance because intra-oral physical wear and the periodic intake of acidic foods or drinks limit the time for remineralisation. Clearly, there is a need for more data on remineralisation or rehardening times for enamel eroded by extrinsic acids. The aim of the current study in vitro was to determine the effect of time on the surface rehardening of enamel placed in artificial saliva after citric acid erosion using an ultrasonication and profilometry method [Eisenburger et al., 2000, 2001]. Scanning electron microscopy (SEM) was used to follow the surface morphological changes of the enamel samples.

Materials and Methods

Sample Preparation

A detailed account of the materials and methods, for sample preparation, have been given in previous publications [West et al., 1998, 1999; Hughes et al., 1999], so a summary is provided. Enamel samples were cut from buccal and lingual surfaces of unerupted human third molar teeth and were embedded in epoxy resin producing specimens measuring 8×5×2 mm. The samples were polished with 800-grit abrasive paper to produce a flat exposed enamel surface with a mean surface profile within ±0.3 μm measured across two zones by a profilometer (Planer Products Ltd., Sunbury-on-Thames, UK). The samples were covered with PVC tape to leave an exposed window of enamel approximately 2 mm wide [Eisenburger et al., 2000].

Experimental Procedure

Ninety-one enamel samples were divided into seven groups of 13 specimens. Six groups were designated the test groups and one the control group. From each group 3 specimens were randomly selected for post-treatment scanning electron microscopic observation. These samples went through the allotted treatments, but no profilometric measurements were performed. All specimens were eroded for 2 h at 35°C in 0.3% citric acid adjusted to pH 3.2 with sodium hydroxide. During erosion the solution was stirred slowly. After erosion all samples were rinsed under running tap water. The tape was removed and two profilometric measurements were taken across the exposed enamel window. All samples were then retaped.

The specimens in the control group were ultrasonicated stepwise up to 480 s. Specimens were placed into 70 ml saline at 20±2°C in a plastic beaker sited in an ultrasonic bath (Pul 55, Kerry Ultrasonics Ltd., Hitchin, UK) containing 300 ml tap water. The ultrasonic bath had a power output of 100 W at 38 kHz. After ultrasonication for 5, 30, 120, 240 and 480 s, profilometric measurements were repeated.

The specimens of the six test groups were placed in artificial saliva (0.7 mmol/l $CaCl_2$, 0.2 mmol/l $MgCl_2$, 4.0 mmol/l KH_2PO_4, 30.0 mmol/l KCl, 20.0 mmol/l Hepes, pH 7.0) for 1, 2, 4, 6, 9 or 24 h at 35°C. During remineralisation the solution was stirred slowly. After the allocated time, each group of samples was removed, rinsed under running tap water and new profilometric measurements were recorded. Afterwards the test samples were ultrasonicated and measured as described above for the control group.

Scanning Electron Microscopy

The additional test and control specimens not used for profilometric measurement were air-dried, sputter-coated with gold and examined at 25 kV in the scanning electron mode in a S90 SEM (Cambridge Instruments). Photomicrographs were taken after incubation in artificial saliva and after 5 and 480 s of ultrasonication.

Statistics

For each stage of the experiment, the mean of the two profilometric measurements was used as the outcome value of each sample. The lesion depth of each sample after storage in artificial saliva was used as the new baseline for the calculation of the changes caused by the ultrasonication process. Statistical analysis was performed using analysis of variance and if significant at $p<0.05$, the Tukey test was used to compare the different groups.

Results

Profilometric Investigation

After 2-hour erosion, the mean erosion depth was 20.4 μm (SD 3.5 μm) calculated over all specimens. Storage in artificial saliva resulted in a small reduction of the depth. The values of the 24-hour group were reduced by 1.1 μm (SD 1.2 μm), of the 9-hour group by 0.6 μm (SD 0.9 μm), of the 6- and 4-hour group by 0.9 μm (SD 0.8 and 0.7 μm), of the 2-hour group by 0.8 μm (SD 1.0 μm) and of the 1-hour group by 0.7 μm (SD 1.1 μm). These changes were statistically significant for the 24-, 6-, 4- and 2-hour groups ($p<0.05$).

The changes in depth resulting from ultrasonication process are shown in figure 1. The control group without any remineralisation had a further substance loss of 2.3 μm (SD 0.6 μm) after 5 s of ultrasonication and of 2.9 μm (SD 1.2 μm) after 30 s. Remineralisation for 2 h reduced the loss to 1.2 μm (SD 1.1 μm) and 1.7 μm (SD 1.3 μm), respectively. After 6-hour remineralisation, the mean loss after 5 s was 0.21 μm (SD 0.42 μm) and 0.47 μm (SD 0.57μm) after 30 s. After 9-hour remineralisation, ultrasonication caused a loss of only 0.02 μm (SD 0.25 μm) after 5 s and 0.06 μm (SD 0.30 μm) after 30 s. Interestingly, ultrasonication for 5 s of the 24-hour group led to a mean reduction in erosion depth, i.e. an apparent mean gain in mineral of 0.4 μm (SD 0.08 μm). Longer ultrasonication times, however, resulted in small mean losses and the original depth was re-established after 240 s. Analysis of variance showed overall statistically significant differences between the groups ($p<0.01$). The Tukey-HSD test revealed three homogeneous groups. Thus, no significant differences were found between the control group and the 1- and 2-hour remineralisation group ($p>0.05$). The 4-hour group was significantly different to all other groups ($p<0.05$) and no significant

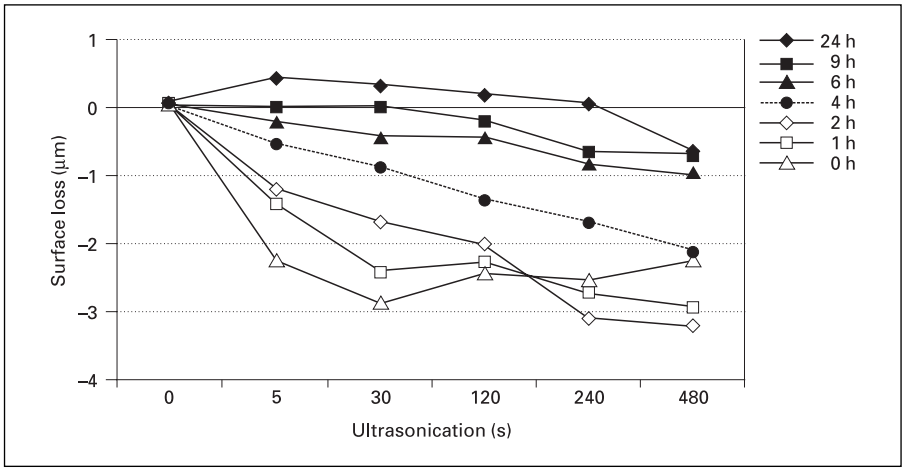

Fig. 1. Surface loss during ultrasonication of eroded specimens which had been remineralised for 0–24 h.

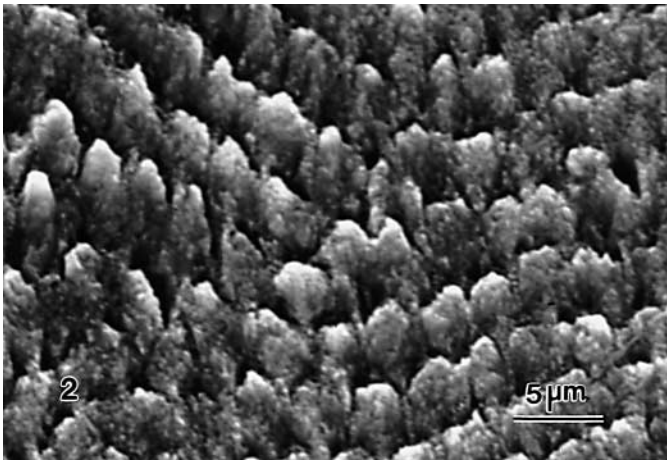

Fig. 2. Surface structure after 2-hour erosion and 5-second ultrasonication. Enamel prisms are visible.

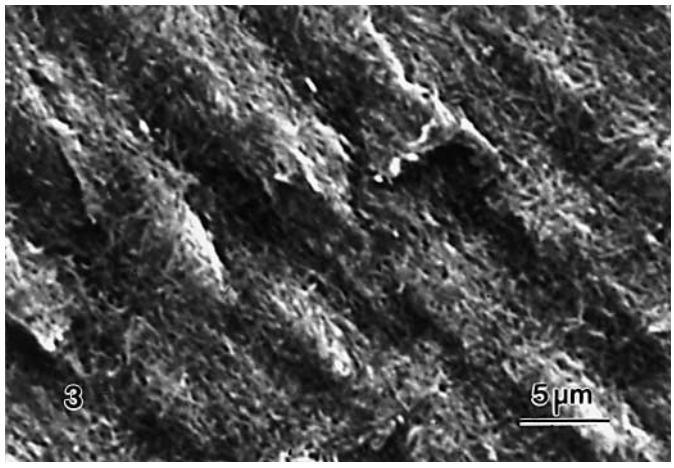

Fig. 3. Surface structure after 2-hour erosion and 1-hour remineralisation showing an amorphous mineral deposition.

differences could be shown between the 6-, 9- and 24-hour test groups ($p > 0.05$). Furthermore, analysis of the three homogeneous groups, namely the control, 1- and 2-hour group, the 4- and 6-hour group and the 9- and 24-hour group revealed that they were significantly different from each other ($p < 0.05$).

SEM Investigation

The control specimens had an amorphous layer on top of a prismatic structure after the erosion process [Eisenburger et al., 2001, fig. 2], but ultrasonication for 5 s removed this deposit to reveal the enamel prisms (fig. 2). After remineralisation SEM observation showed an amorphous mineral deposition on top of underlying enamel prisms (fig. 3). Some samples presented a continuous layer, while others had a more structured surface deposit of mineral, enclosing the enamel prisms. After a short remineralisation period, ultrasonication for 5 s resulted in an alteration of the surface structure. Thus 1-hour specimens showed a partial loss of the mineral deposit, revealing a deeper layer (fig. 4). After 2-hour remineralisation, the mineral deposit appeared more crystalline after 5-second ultrasonication (fig. 5). Ultrasonication for 480 s led to complete removal of the mineral layer revealing flattened enamel prisms (fig. 6). Samples remineralised for 6 h or more retained a mineral deposit on top of the enamel prisms, even after 480-second ultrasonication (fig. 7).

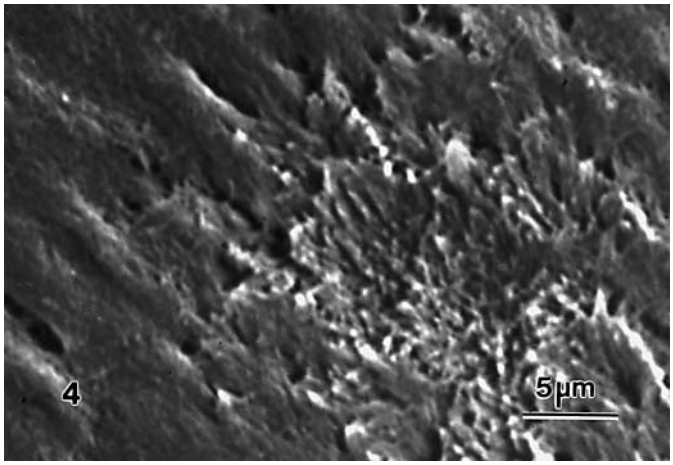

Fig. 4. Surface structure after 2-hour erosion, 1-hour remineralisation and 5-second ultrasonication.

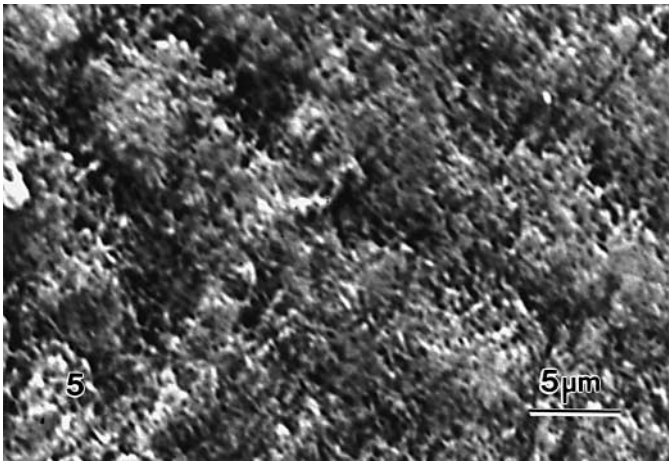

Fig. 5. Surface structure after 2-hour erosion, 2-hour remineralisation and 5-second ultrasonication.

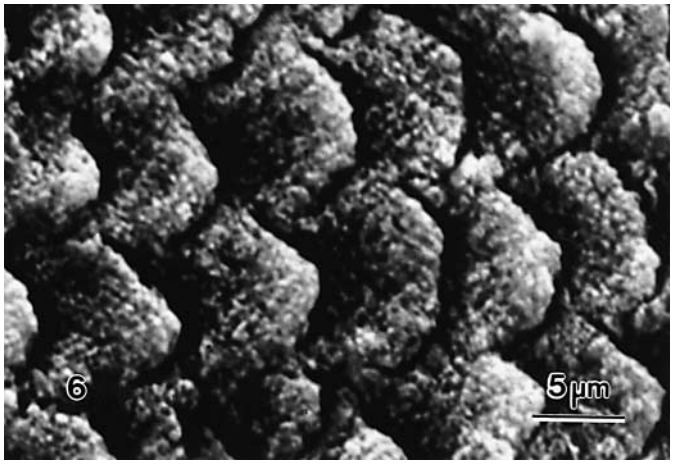

Fig. 6. Surface structure after 2-hour erosion, 2-hour remineralisation and 480-second ultrasonication. A partially flattened prismatic structure can be seen.

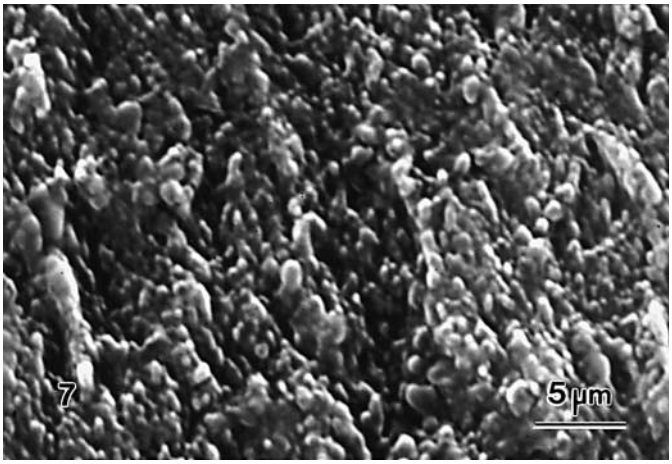

Fig. 7. Surface structure after 2-hour erosion, 6-hour remineralisation and 480-second ultrasonication. The mineral deposition is still present.

Discussion

Comparison of profilometric measurements before and after remineralisation showed a reduction in depth ranging from 0.6 to 1.1 μm. This can be explained by either a mineral precipitation on top of the softened surface or within the softened enamel layer. It has been shown that most of the non-remineralised and thereby softened enamel can be removed by ultrasonication during the first 30 s [Eisenburger et al., 2000]; thereafter only little change was noted. In the current study, the further loss of enamel in the control group was 2.3 μm after 5-second and 2.9 μm after 30 second ultrasonication. The remineralisation process reduced the substance loss of all test groups at the 5- and 30-second time points, although some effect was noted after a longer ultrasonication period. In particular, the 4-hour group showed a nearly linear increase in depth with ultrasonication time up to 480 s. This suggests that the remineralisation reduces the initial loss and causes a time delay for further loss, presumably because the softened zone has been partially rehardened.

A complete stabilisation or hardening of surface-softened enamel after 24-hour remineralisation in artificial saliva was demonstrated in a previous laboratory investigation [Eisenburger et al., 2001] and was shown again in the present study. The current study was conceived in an attempt to

identify at which time point within the 24-hour period complete remineralisation occurred. The data indicate that the remineralisation process was complete after 6 h, at least with regard to the mechanical stabilisation of softened enamel against ultrasonication. Although there were small differences in the mean values between the 6-, 9- and 24-hour groups, they were not statistically significant. The remineralisation process, however, did not restore the original surface structure of sound enamel. In line with other studies, remineralisation is not a restitutio ad integrum with prism regrowth [Mühlemann et al., 1964; Imfeld, 1996] but a repair process by mineral deposition into the porous zone [Mühlemann et al., 1964; Collys et al., 1993; Imfeld, 1996]. It has been suggested that the amorphous mineral layer observed on the surface of the control specimens, after erosion, was a reprecipitation of calcium phosphate when specimens are transferred from the acid to water, with the concomitant rapid change in pH [Boyde et al., 1978; Eisenburger et al., 2001]. This layer can be removed easily by ultrasonication and is accompanied by a flattening of the underlying enamel prisms [Eisenburger et al., 2001]. However, mineral deposition from the artificial saliva seems to be much more resistant to ultrasonication. After 1- and 2-hour remineralisation, the deposit was lost only partially after 5-second ultrasonication but completely after 480-second ultrasonication (fig. 6). Nevertheless even at this stage the prisms seem to have greater relief than without remineralisation. This would be consistent with the profilometric measurements, which showed a smaller surface loss in the 1- and 2-hour groups than in the control group. However, it appeared that only after a remineralisation period of 6 h or more, the mineral deposited was sufficient to withstand ultrasonication. Also the measurements indicate only an enamel loss of less than 1 μm after 480 s.

In conclusion, this study suggests that exposure to artificial saliva re-stabilises softened enamel, in that this zone can no longer be removed by ultrasonication. Although there is a trend to a smaller substance loss following longer remineralisation times, no statistically significant differences could be shown. Shorter remineralisation times appear to provide only partial rehardening of softened enamel. Accepting that it is difficult to extrapolate findings in vitro to effects in vivo, these data suggest that enamel softened by acids entering the oral cavity is very susceptible to physical insult for some time after the erosive event. Clearly, there is a need for more data from studies in vivo using classical randomised controlled clinical protocols, ideally with a crossover design and using the established methodologies to study erosion in situ [West et al., 1998].

References

Arends J, ten Cate JM: Tooth enamel remineralisation. J Cryst Growth 1981;53:135–147.

Arends J, Gelhard TBFM: In vivo remineralisation of human enamel; in Leach SA, Edgar WM (eds): Demineralisation and Remineralisation of the Teeth. Oxford, IRL Press, 1983, pp 1–16.

Attin T, Buchalla W, Gollner M, Hellwig E: Use of variable remineralisation periods to improve the abrasion resistance of previously eroded enamel. Caries Res 2000;34:48–52.

Attin T, Deifuss H, Hellwig E: Influence of acidified fluoride gel on abrasion resistance of eroded enamel. Caries Res 1999; 33: 135–139.

Boyde A, Jones SJ, Reynolds PS: Quantitative and qualitative studies of enamel etching with acid and EDTA. Scanning Electron Microsc 1978; 2:991–1002.

Collys K, Cleymaet R, Coomans D, Michotte Y, Slop D: Rehardening of surface softened and surface etched enamel in vitro and by intraoral exposure. Caries Res 1993;27:15–20.

Eisenburger M, Hughes J, West NX, Jandt KD, Addy M: Ultrasonication as a method to study enamel demineralisation during acid erosion. Caries Res 2000;34:289–294.

Eisenburger M, Hughes J, West NX, Shellis RP, Addy M: The use of ultrasonication to study remineralisation of eroded enamel. Caries Res 2001;35:61–66.

González-Cabezas C, Fontana M, Dunipace AJ, Li Y, Fischer GM, Proskin, HM, Stookey GK: Measurement of enamel remineralization using microradiography and confocal microscopy. Caries Res 1998;32:385–392.

Hughes JA, West NX, Parker DM, Newcombe RG, Addy M: Development and evaluation of a low erosive blackcurrant juice drink in vitro and in situ 1: Comparison with orange juice. J Dent 1999;27:285–289.

Iijima Y, Takagi O, Ruben J, Arends J: In vitro remineralisation of in vivo and in vitro formed enamel lesions. Caries Res 1999;33:206–213.

Imfeld T: Prevention of progression of dental erosion by professional and individual prophylactic measures. Eur J Oral Sci 1996;104:215–230.

Jaeggi T, Lussi A: Toothbrush abrasion of erosively altered enamel after intraoral exposure to saliva: An in situ study. Caries Res 1999;33:455–461.

Klimek J, Hellwig E: Beeinflussung der De- und Remineralisation von Zahnschmelz durch Zähneputzen mit einem Zahnsalz. Oralprophylaxe 1989;11:26–30.

Mühlemann HR, Lenz H, Rossinsky K: Electron microscopic appearance of rehardened enamel. Helv Odontol Acta 1964;8:108–111.

West NX, Hughes JA, Parker DM, Newcombe RG, Addy M: Development and evaluation of a low erosive blackcurrant juice drink. 2. Comparison with a conventional blackcurrant juice drink and orange juice. J Dent 1999;27:341–344.

West NX, Maxwell A, Hughes JA, Parker DM, Newcombe RG, Addy M: A method to measure clinical erosion: The effect of orange juice consumption on erosion of enamel. J Dent 1998;26:329–335.