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### Remineralization of human enamel in vivo.

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*Document Version*

Publisher's PDF, also known as Version of record

*Publication date:*

1982

[Link to publication in University of Groningen/UMCG research database](#)

*Citation for published version (APA):*

Gelhard, T. B. F. M. (1982). *Remineralization of human enamel in vivo*. s.n.

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## CHAPTER 8

### SUMMARY

Dental caries is a major disease in modern society. In the past dental caries has been considered mainly as a demineralization process causing firstly loss of mineral through an intact surface layer and ultimately leading to cavitation.

In the last decade some investigators showed that demineralization could be *reversed* (remineralization).

Recent studies demonstrated conclusively that dental caries is actually a situation of unbalance between de- and remineralization.

Aim of the investigation was firstly to study remineralization *in vivo* longitudinally over a period of 3 months.

Two different enamel *defects* have been studied:

1. subsurface enamel lesions.
2. surface softened enamel (an initial stage of lesion formation).

A second aim was to investigate in detail the enamel properties *after* the 3 month remineralization period, and to attempt to correlate remineralization and salivary properties.

Human enamel specimens were placed in the prostheses of 30 patients for 3 months. The patients were divided in three groups: a brush (tooth paste 1500 ppm  $F^-$ ), a rinse (50 ppm  $F^-$ ) and a control group.

In chapter 1, de- and remineralization are introduced and described briefly. A schematical presentation is given of the 3 enamel defects most interesting from the view point of remineralization: subsurface lesions, surface softened enamel and etched enamel.

In the second chapter a survey of the literature on remineralization is given; important *in vitro* data are summarized briefly.

This chapter shows two important aspects:

1. in vitro and in vivo remineralization are most likely *very different*.
2. previously published clinical remineralization studies give either DMFS values (but no information on the remineralization phenomena) or, in experiments using enamel in prostheses limited information, because only a few patients were used (no statistical analysis).

In chapter 3 methods and materials are described. Specimen preparation, fixation of the specimens in the prostheses as well as patient selection and patient instructions are presented in detail.

The experimental techniques employed are microhardness indentation measurements, microradiography (MR), secondary ion mass spectrometry (SIMS) especially for F determination, infra red spectroscopy (IR) and scanning electron microscopy (SEM).

Indentation measurements were done on sound and demineralized enamel at the beginning of the experiment ( $t = 0$ ). Remineralized enamel was measured at  $t = 2, 4, 16, 30, 33, 58$  and  $87$  days. Finally at  $t = 87$  the sound enamel value was determined again.

After 3 month SEM, IR, MR and SIMS measurements were done on the in vivo remineralized specimens.

Furthermore the methods for the determination of pH, buffer capacity, total calcium, free calcium and total phosphate content in the patients saliva are described.

Chapter 4 gives the results of lesion remineralization as measured by means of microhardness measurements.

In the first part examples of lesion remineralization of individual patients are presented. The remineralization sometimes fluctuated strongly in one individual during the 3 month period. Two enamel specimens positioned in one mouth do remineralize sometimes quite different.

In the second part data averaged over 20 enamel specimens (patients) are given and discussed.

The main conclusions from this chapter are:

1. Subsurface lesions are remineralized in vivo *very slowly* compared to in vitro remineralization, the in vivo remineralization process is orders of magnitude slower.

2. In the remineralization process saliva plays an important role.
3. After 3 month in vivo remineralization the rehardening effects of the 3 groups are:  
*brush group(C) = control group(A) > rinse group(B).*
4. In the brush and control group there is a more continuous remineralization than in the rinse group.
5. The remineralization rate is relatively fast in the first two weeks. For longer periods the remineralization rate is extremely slow.

In vivo remineralization results on surface softened enamel are described in chapter 5. The set-up of chapter 5 is comparable to that of chapter 4. In addition, material loss values due to enamel-patient interaction or due to brushing (or rinsing) are investigated for all 3 groups.

The main conclusions from chapter 5 are:

1. Softened enamel remineralization in vivo is relatively fast as compared to subsurface lesion repair.
2. Softened enamel is with respect to subsurface lesions more completely repaired.
3. After 3 month the in vivo rehardening effects are:  
*brush group(C) >> control group(A) > rinse group(B).*
4. Surface softened enamel remineralization differs from patient to patient and from specimen to specimen.
5. The rehardening rate of the control group is very small. The rehardening rate of the brush group is fast up to 1 month; later on the rate is  $\approx 1$   $\mu$ m indentation length reduction in 8 days. Assuming linearity to be valid at periods over 3 month, one may expect complete rehardening (up to the sound level) in  $\approx 4$  month. For the rinse group a similar extrapolation can be made. Assuming linearity full repair may be expected in about 7 month.
6. Material loss due to enamel-patient interaction or due to brushing (or rinsing) is negligible and comparable for all 3 groups investigated.

In chapter 6 the main results of measurements on the enamel after 3 month in vivo remineralization are given. Microradiography is most suitable for the interpretation of mineral deposition as far as lesions are concerned. Mineral deposition of surface softened enamel is (due to the origin of this type of enamel defect) much more different to follow with this technique.

Considering the 3 groups of interest we have (after 3 month):

$$V_{sl} \text{ brush} > V_{sl} \text{ control} > V_{sl} \text{ rinse}$$

in which  $V_{sl}$  is the average volume% of mineral in the surface layer covering the lesion.

For the  $V_1$  values ( $V_1$  is the minimum mineral content in the lesion body we have:

$$V_1 \text{ brush} > V_1 \text{ control} \approx V_1 \text{ rinse.}$$

Calculation of the ratio  $V_{sl}/V_1$  gives for all groups a constant value of 1.1 (after 3 month).

The average lesion depth ( $d_1$ ) of the 3 groups are after the in vivo experiment:

$$d_1 \text{ brush} \approx d_1 \text{ rinse} \approx d_1 \text{ control (only brush versus control: } p < 0.05).$$

Microradiography gives also the  $d_{sl}$  value ( $d_{sl}$  being the thickness of the surface layer).

For all 3 groups the ratio  $d_{sl}/d_1 \approx 0.4$ .

From the fact that  $V_{sl}/V_1$  and  $d_{sl}/d_1$  are constant we have to conclude that in all 3 groups the remineralization mechanism of mineral deposition is the same; however in case of brushing remineralization is more efficient.

The fluoride distribution data on the in vivo remineralized specimens lead to the following conclusions:

For lesion remineralization:

1. For an efficient remineralization, the acquired fluoride should reach the lesion front.
2. The  $F^-$  gradient (more than the absolute value of  $F^-$ ) determines most likely the remineralization efficiency.
3. The amount of  $F^-$  in the agent (50 ppm rinse and 1500 ppm brush) is not crucial for the amount of  $F^-$  acquired near the enamel surface in a lesion; but is indeed crucial near the lesion front.

For surface softened remineralization in vivo:

1. Very near the outer enamel surface ( $\approx 0.1 \mu\text{m}$ ) we have:

$F^-$  brush >  $F^-$  rinse >  $F^-$  control.

2.  $F^-$  levels in the rinse experiment create a rather high F content 0.2 - 1.0  $\mu$ m from the outer surface.
3.  $F^-$  in the brush group penetrates deep into the softened enamel up to 30  $\mu$ m.

The most important result of the SEM experiments is the observation that the surface roughness present in *surface* softened enamel causes a *local bacterial penetration* in the enamel. This was especially evident in control and rinse group, less in the brush group.

Another result from SEM studies was that in lesion remineralization, the outer surface seems to be relatively porous. Precipitation on the outer enamel surface was not observable.

The IR data show that the IR spectra of sound and remineralized enamel are nearly identical. However, quantitatively both the  $CO_3^{2-}$  and  $HPO_4^{2-}$  contents are roughly 1.5 x larger in remineralized than in sound enamel.

In chapter 7 various salivary data are presented. In the second part correlations with remineralization parameters are described. If one correlates the individual salivary properties with individual remineralization parameters we find linear positive correlations between:

1.  $V_1$  (minimum mineral content in the lesion) and the free  $Ca^{2+}$  content in saliva.
2.  $V_1$  and the salivary pH.
3.  $\alpha$  (the relative microhardness indentation length reduction) and the salivary pH (lesion experiment).
4.  $\alpha$  and the free  $Ca^{2+}$  content. This correlation was found *both* in the lesion experiment and in the surface softening experiment.

In conclusion we can say that:

- a) Enamel defects still in the surface softening stage can be remineralized much faster and more complete than subsurface lesions.
- b) In both experiments (lesions and surface softened enamel) the use of a fluoridated tooth paste was much more efficient in re-

mineralization than rinsing with fluoride.

- c) In the surface softened enamel remineralization experiments, the efficiency of remineralization is always *better* in the control than in the 50 ppm F rinse group.
- d) The fluoride gradient and especially the fact that the  $F^-$  must reach the lesion front is crucial for remineralization efficiency.
- e) Remineralization in vitro and in vivo are *completely different* as far as *efficiency and speed* are concerned.
- f) SEM experiments show that after 3 month in vivo remineralization, the outer surface of lesions is still porous; there is no surface precipitation or bacterial penetration. Surface softened enamel showed often local bacterial penetration.
- g) Remineralization is strongly patient and enamel specimen dependent.
- h) From the salivary point of view the pH and free calcium content are most important parameters in the mineral deposition processes.

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