The inhibition of enamel demineralization under constant composition conditions
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Caries in its various forms is one of the most widespread diseases of mankind. With proper oral hygiene (i.e. plaque removal) and adequate fluoridation caries can often be prevented. Caries in vivo is caused by an acid attack from numerous bacteria in the dental plaque. The strength of the acid attack depends on amount, composition and location of the plaque as well as on the sugar and fermentable carbohydrate intake. Saliva and plaque are supersaturated with respect to hydroxyapatite (HAP). This means that after an acid attack by bacteria (demineralization), remineralization (mineral deposition) can take place in the enamel. This has the practical consequence that in vivo, de- and remineralization periods alternate frequently.

The emphasis in this study is on enamel demineralization. In the past, many demineralization studies have been carried out to study the kinetics and possibly the mechanism of enamel demineralization. Artificial caries-like lesions have been produced in demineralizing solutions with in due time variable concentration conditions. If however, accurate information about physico-chemical processes during demineralization is needed, demineralization conditions should be defined as good as possible, i.e. the activities of the ionic species involved should be kept constant.

In Chapter 1 the two important mineral distributions after enamel demineralization are pointed out: the surface softening type of demineralization without a surface layer and the subsurface demineralization in which a surface layer covers the lesion. Two important types of inhibitors of the enamel demineralization process, fluorides and diphosphonates are briefly discussed.

The aims of this investigation were:
1. To develop an experimental technique suitable for de- or remineralization of enamel under constant-composition conditions.
2. To study the inhibiting effect on enamel demineralization of fluoride (F) and methylhydroxydiphosphonate (MHDP), separately and in combination.
3. To follow mineral distributions in enamel DURING demineralization with special emphasis on surface layer formation.

Chapter 2 of this thesis presents a literature survey on dental enamel, plaque, initial caries and demineralization in vitro. From this literature survey it is shown that most demineralizing systems employed for enamel demineralization have variable concentration conditions. They have changing supersaturations and are sometimes inapt for studying the kinetics and mechanisms of enamel demineralization.

It is shown that a potentiostatic constant-composition method, (though useful for apatite growth studies, or other crystal growth studies), is not very suitable for studies of enamel demineralization.

From a brief state of the art on inhibitors it is concluded that fluoride is probably the only inhibitor having a negative inhibiting effect on crystal growth of hydroxyapatite (i.e. positive effect on crystal growth) and a positive inhibiting effect on crystal dissolution of hydroxyapatite (i.e. negative effect on crystal dissolution).

From literature data, it is concluded as well that at moderate and low levels of fluoride in demineralizing solutions (< 0.5 ppm) the lesion depth of demineralized enamel is nearly independent from the fluoride concentration. However, mineral loss (ΔZ), dissolution rate and morphology of enamel are strongly dependent on the presence and concentration of fluoride.

A new apparatus suitable for de- and remineralization of enamel in vitro under constant-composition conditions is described in Chapter 3. During artificial lesion formation (using an acetate buffer containing methylhydroxydiphosphonate, MHDP, as inhibitor) the composition of the solution is constant. The measured Ca and phosphate variation is less than 3%, the pH variation less than 0.03 pH unit. For enamel these variations are within the biological variation between enamel specimens.

Experimentally it was found that the liquid streaming over the enamel surface does not measurably remove material from the surface (erosion) within an experimental error of 0.1 μm. Thus the outer enamel surface does not change position with respect to the sound enamel surface during demineralization.

A few examples of the usefulness of the constant-composition method for de- as well as remineralization are presented. In the demineralization experiment it is shown by means of microradiography, microhardness measurements
with light microscopy, that the method quickly produces reproducible homogeneous demineralization of bovine enamel.

In a remineralization experiment of demineralized enamel effective remineralization occurs in 360 h. In this experiment a threefold increase in microhardness and a twofold decrease in lesion depth with respect to the starting situation was found. Effective remineralization is obviously not influenced by the low concentrations of MHDP present during demineralization (high concentrations of MHDP present are known to inhibit remineralization).

The effect of various MHDP concentrations on demineralization was investigated in the range of 2-50 μM. The results show that even low concentrations of MHDP during demineralization prove to be effective in lesion depth reduction.

In Chapter 4 a new microradiographic approach for measuring the mineral content after various demineralization stages of the same enamel section and of the same area (3x30x120 μm³) is introduced. A special computer program directs the densitometer always to a previously defined spot; repositioning errors are smaller than 3 μm. The method described uses thick sections (up to 300 μm can be used). The errors in mineral content are calculated to be of the same order (3% by volume) for thick sections as for thin sections. The experiments with this special single section technique showed that the first stage in enamel demineralization is a surface softening process; later on a surface layer forms. Consecutive tracings for a demineralization experiment for prolonged demineralization even shows alternating dissolution and reprecipitation processes in the surface layer region.

A linear least-square fit of the cube of the lesion depths versus demineralizing period show a high correlation coefficient (R=0.99). This is also valid for the cube of the microhardness indentation lengths made perpendicular to the surface and the demineralization period (R=0.91). There is also a linear correlation (R=0.96) between lesion depth and indentation length, indicating that once this correlation is established for a given system, indentation measurements are an easy method to follow demineralization.

Chapter 5 deals with the effect of fluoride and MHDP alone and in combination on the enamel demineralization under constant-composition conditions. It is shown that the calculated increase of Ca and phosphate concentrations...
in solution is less than 2.5% and 1.5% respectively. The driving force is constant within 2.6%.

Low and high levels of inhibitor concentrations as well as a combination of inhibitors were investigated. At low concentrations (11.6 µM F and 6 µM MHDP) both inhibitors as well as the combination of inhibitors gave subsurface demineralization. The inhibiting effects of fluoride and MHDP were at the concentrations mentioned comparable. The combined effect of the two inhibitors appears to be less than the effect expected. This is most likely due to the fact that the inhibitors are competitive.

The mineral content in the enamel surface layer after demineralization with a low inhibitor content ranks as:

\[ F > MHDP > F + MHDP. \]

At high inhibitor concentrations (116 µM F and 60 µM MHDP) both fluoride only and fluoride plus MHDP combined gave total inhibition of the enamel demineralization.

A high concentration of 60 µM MHDP only, resulted in a decreased but measurable subsurface demineralization.

Inhibitors adsorb on the surface of hydroxyapatite crystals in enamel, and/or complex with ions in solution. This phenomenon is described in Chapter 6. In general the amount of enamel dissolved and transported from the lesion to the enamel surface is the rate controlling process. In this diffusion determined process lesion depths \( l_d \) cubed varies linear with time \( l_d^3 = \alpha t + q \).

At higher concentrations of inhibitor the dissolution process takes place on the surface of enamel crystallites; in that case the slow surface dissolution is the rate-controlling process \( l_d = \alpha t + q \). At very high inhibitor concentrations the inhibitor complexes with lattice ions in the aqueous solution in the lesion, again diffusion is the rate-controlling process \( l_d^3 = \alpha t + q \).

For good inhibition the inhibitor must be able to interact with the ions in the crystallite surface.
Large formation constants for complexes between the inhibitor and ions in the solution, result in non-reactive complexes in the solution and poor inhibitors.

A simple model assuming complexation of the inhibitor with calcium in the solution (association constant $K_r$) and assuming Langmuir adsorption of the inhibitor onto the crystals (adsorption constant $K_L$) shows that if $K_r$ is large and/or $K_L$ is small, good inhibition is not possible.

In this chapter three criteria are given for ideal inhibition of enamel dissolution.

This thesis leaves several questions open for discussion. The data of the combined effect of fluoride and MHDP on enamel demineralization could not be completely interpreted. Furthermore results on enamel inhibition of bulk enamel do not agree with those obtained from powdered enamel studies. Different inhibitors and different combinations of inhibitors should be tested in the future.