Methods for the evaluation of caries preventing agents
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Dental caries, or tooth decay, is a pathological process of localized destruction of tooth tissues by micro-organisms present in the so-called plaque. The destruction, caused by acids formed in the plaque, leads to mineral loss (demineralization) from the hard tissues. A demineralized tooth can also gain mineral, due to a process called remineralization. A tooth is clinically "stable" when demineralization and remineralization are in equilibrium.

To maintain or restore the equilibrium situation, several caries preventive measures can be taken. The severity of the attack can be reduced by "changing" the plaque substrate by the consumption of less cariogenic food, by reduction of the sweet intake frequency (periods of demineralization are followed by longer remineralization periods) and by plaque reduction. These preventive measures are very effective in caries reduction, but ask for a considerable patient compliance, which is often difficult to maintain.

To make caries prevention less dependent on the patients' compliance, the tooth itself can be protected by several means, for example by fluoride. Fluoride is widely used as active ingredients in caries preventing agents: for example in dentifrices, mouthwashes, tablets or topical applications etc.

From the clinical point of view, the beneficial effect of caries-preventing agents should be evaluated under conditions which correspond as closely as possible to the "real" situation.

In this thesis, the effect of fluoride on de- and remineralization of enamel and dentine is considered; several In vitro and in situ tests are evaluated and attuned to produce an integrated approach, which contributes in
Firstly, several methods to measure mineral changes in tooth material are evaluated. In Chapter 2 surface micro-hardness measurements, which are pseudo non-destructive, are evaluated for their potential use on dentine. Microhardness indentations in dentine show relaxation behavior with time. In a comparison between indentation length and mineral concentration it is shown that for human dentine no direct relation exists between the hardness indentation length and the mineral content of the samples. It is concluded that surface hardness measurements are not applicable for the non-destructive assessment of mineral changes in dentine, during de- or remineralization.

In chapter 3, the theoretical base for Wavelength-independent Microradiography (WIM), is described and tested. To test the wavelength independency of the method, mineral concentrations of flattened enamel and dentine samples with a thickness up to 500 μm are determined for sound and for demineralized samples at 20 and at 60 kV X-ray tube voltage. It is shown, that a mineral quantification is reached within 1.5%, independent of the x-rays used. In a comparison of mineral concentrations measured by WIM and by Longitudinal Microradiography (LMR), a correlation coefficient of 0.99 is found for enamel and 0.96 for dentine. It is concluded, that the new method is potentially suitable for non-destructive mineral assessment of whole teeth.

In Chapter 4, WIM is checked on its applicability to follow mineral changes during demineralization of whole teeth. This is done in three steps with increased complexity: In the first step natural, curved surfaces are investigated. The mineral content of about 0.3 mm thick enamel and dentine samples with natural surfaces is determined by WIM and by LMR. A correlation between both methods of 0.98 is found for both the enamel and the dentine samples. In the second step thick tooth sections are simulated by adding a block of
5 mm dentine to thin enamel and dentine samples. Mineral measurements with WIM of the samples plus the dentine block are compared with mineral measurements of the thin samples (without block) for different stages of mineralization. A correlation of 0.97 between measurements with- and without block is found for the enamel and 0.90 for the dentine samples. Finally, in a third step, the demineralization of whole premolars is followed.

In the second part of this thesis, several in vitro testing methods are evaluated. In chapter 5, in vitro demineralization methods are compared. A new pH-cycling system, in which the employed de- and remineralization solutions have a constant composition, is presented. It is used to compare the amount of mineral lost from human dentine and enamel with and without their natural surfaces when small amounts of fluoride are added to a demineralizing solution. Also several different demineralization/remineralization ratios - in the range 1:1 to 1:4 - are investigated. A linear correlation is found between the amount of mineral lost and the total demineralization period for both dentine and enamel. For both tissues, the removal of their outer surface roughly doubles the amount of mineral lost. Under the used pH-cycling conditions, a logarithmic relation between mineral loss and concentration of added fluoride was found. About 2 ppm $F^-$ is needed to inhibit the enamel demineralization completely; in the case of dentine much more $F^-$ in solution is necessary to achieve inhibition.

In the first part of Chapter 6 the pH-cycling apparatus mentioned is extended to a fully automated and standardized apparatus, which imitates the oral situation by pH-cycling and brushing with a dentifrice. It is applied to test the effect of 5 dentifrices on the demineralization of pre-demineralized enamel in vitro. The conditions are such that demineralization dominates. Mineral loss decreases linearly with the total amount of fluoride in the dentifrices ($r=0.95$). All dentifrices but one significantly reduce the
amount of mineral loss compared with the control dentifrice. The dentifrice containing 1130 ppm NaF decreases demineralization significantly more compared with the products containing mixtures of NaF and MFP or the placebo. No statistically significant difference is found between the product containing NaF only and the product containing NaF and pyrophosphate (P<0.05). In the last part of this work, several *in situ* methods to test the efficacy of caries preventing agents are evaluated.

In the second part of Chapter 6, an *in situ* model using abraded enamel samples fixed in a denture is employed to compare the effect of the 5 dentifrices *in situ* on plaque-induced mineral loss. *In situ*, the experiments show that no effective remineralization occurs during brushing with the dentifrices for a period of 9 weeks. The total amount of fluoride in the dentifrices and mineral loss were only weakly related (r=0.58). Again, the dentifrice containing 1130 ppm NaF decreases demineralization significantly more compared with the products containing mixtures of NaF and MFP or the placebo. Again no statistically significant difference is found between the product containing NaF only and the product containing NaF and pyrophosphate (P<0.05).

Because the model described above is limited to investigations pertaining to smooth surface enamel demineralizations, a new *in situ* model is proposed in Chapter 7. In this model enamel and dentine demineralizations can be studied both on the smooth surfaces and on the approximal sides of whole teeth. Using Wavelength-independent Microradiography changes, in the enamel and dentine of the premolars can be monitored. The model is currently being used to compare the mineral loss or gain effects caused by a dentifrice containing 1130 ppm NaF on enamel and dentine smooth surfaces with the effect on approximal sides.
Finally, in Chapter 8, the new *in vitro* and *in situ* methods are combined with the new mineral assessment method. Suggestions for further research are given to come to an integrated approach to the evaluation of caries preventing agents.