Antimicrobial varnish and root surface caries
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SUMMARY

The original intent of this thesis was to determine the potential inhibitory effect of an antimicrobial-releasing varnish on root surface demineralisation in situ. This varnish (Cervitec*) was designed in order to deal with a major caries problem in clinical dentistry today and in the future: root surface caries. Previously published studies on antimicrobial-releasing varnishes have been focussed in particular on the microbiological aspects of plaque- and specific bacteria reductions. No quantitative microradiographical data have been reported on the efficacy of antimicrobial varnishes on dental hard tissue demineralisation. Initially, the efficacy of the varnish on dentin was investigated. After promising results, the efficacy on enamel was incorporated in this work.

Primary aims of this thesis, as described in chapter 1, were:
- To study the efficacy of an antimicrobial-containing varnish in situ on root surface demineralisation.
- To investigate the release of the two antimicrobials, chlorhexidine (1% w/w) and thymol (1% w/w) from this varnish system.

Secondary aims were:
- To study the efficacy of the varnish in situ on enamel demineralisation.
- To investigate and describe the adsorption of chlorhexidine to dental hard tissues.

Chapter 2 gives a survey of the literature pertaining to root surface caries, antimicrobial agents, varnishes and a brief discussion of enamel caries. Root surface caries is predominantly confined to teeth with exposed roots, a situation which is in most cases the result of periodontal breakdown. After inadequate oral hygiene and frequent sugar intake, pathogenic plaque action will cause periodontal inflammation, which induces gingival recession, alveolar bone loss and ultimately leads to accessible roots. Root caries primarily affects mature people and its frequency is increasing due to increased life expectancy and dentition retention. The histopathology of root caries is still partly unknown because of the complex de- and remineralisation processes which occur in dentin. Bacteria implicated among others in root caries are mutans streptococci, Actinomyces spp. and also Lactobacillus spp.

Chlorhexidine, though one of the strongest antimicrobials used in the oral cavity, is widely employed because of its broad spectrum activity against gram-negative but especially against gram-positive bacteria. Mutans streptococci seems to be particularly sensitive. Negative side-effects of regular use of high concentrations of chlorhexidine are dark staining of teeth and mucosa, alterations in taste sensation and lesions (desquamations) on the oral mucosa.

Thymol is the other well accepted antimicrobial incorporated in the varnish used in this work and is reported to have a minor in vivo antimicrobial efficacy. Dental varnishes have been previously used as fluoride carriers. Varnishes have the advantages of low dosages of active ingredients, a localized activity (at the site were it is needed) and a prolonged contact time. The antiplaque effects of antimicrobial varnishes have been recently investigated. These studies demon-
strate that the chlorhexidine-containing varnishes had a strong inhibiting effect especially on mutans streptococci and showed little negative side-effects.

In chapter 3 root surface demineralisation in situ is presented after varnish applications. The varnish, with or without active ingredients chlorhexidine and thymol, was applied once or twice on the root surface prior to a two-week in situ demineralisation period. Ten participants carried sound, intact root samples in lower dentures for 4 consecutive 2-week periods in a randomized cross-over design. The application of varnish containing active agents resulted in a statistically significant demineralisation reduction. Reductions of about 80% for lesion depth and mineral loss were measured. The varnish base (without active agents) had no effect.

The efficacy of the varnish on root surface demineralisation as a function of the demineralisation period is presented and discussed in chapter 4. The results show that the caries reduction in the presence of the varnish was significant up until 2 weeks, but decreased in magnitude with the length of the in situ demineralisation period. After 4 and 6 weeks there were no longer statistically significant differences with respect to no treatment. This could be due to the severe demineralisation conditions in this intra-oral model.

The results of the experiments described in chapters 3 and 4 raised the question whether the varnish would also be effective in enamel caries reduction. In chapter 5 the same experimental set-up as presented above was used with enamel. The results show a statistically significant reduction in the lesion depth (reduction of about 50%) for up to 4 weeks while the mineral loss was reduced up to 2 weeks (reduction of about 60%). Differences between lesion progress in dentin and in enamel are discussed. Demineralisation of dentin occurs at twice the rate seen in enamel. Lesion depth and mineral loss in enamel were in situ proportional to the demineralisation period in contrast to prior in vitro investigations.

In chapter 6 the in vitro release of chlorhexidine and thymol from the varnish system is described. U.V. spectrophotometry was employed as measuring technique, using a 2 component analysis for the varnish system containing both antimicrobials. The results show that:
1) agent release takes place for at least 3 months.
2) the amount of chlorhexidine diffusing out of the component varnish is dependent on the presence of thymol.

In chapter 7 the adsorption of chlorhexidine on powdered dentin, enamel and hydroxyapatite (HAP) as well as on intact dentin and enamel is presented. The maximum binding capacity (B_{max}), affinity constant (K) and the fraction of covered adsorption sites is estimated and discussed assuming a Langmuir adsorption model. For powdered substrates the chlorhexidine uptake can be ranked as: dentin >> enamel >> HAP and in intact substrates dentin >> enamel.

The affinity constant K is ± 35 L.g^{-1} in powdered and intact dentin. Furthermore, K (dentin) is smaller than K (enamel) and K(HAP). For powdered dentin and enamel respectively.

It is concluded that:
1) chlorhexidine
2) chlorhexidine

In chapter 5 the results of studies on adsorption are discussed.
1) Differences between in vitro and in situ conditions can be explained in terms of limited information and costs, accuracy and applicability. Presently, these results are under consideration.
2) Efficacy of the varnish base.

Results of a clinical trial are described in chapter 6. It is confirmed that the varnish is effective in reducing lesion depth and mineral loss.

3) The mechanism of action can be considered to be multi-factorial and involves both inhibition of bacterial growth and inhibition of matrix degradation.

4) Clinical evaluation of the varnish is considered.

From this the varnish can be effectively reduce caries in both dentin and enamel and is most likely placed in dentin.
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conditions obtained in a large group of the population. Disadvantage is the
limited information provided. Advantages of in situ experimentation are the low
costs, accurate measuring techniques, the possibility of creating known demi-
neralisation or remineralisation conditions and the use of adequate statistics.
Presently, there are no reasons to assume that the efficacy of the plaque situation
under consideration is very different in in situ and in in vivo conditions.
2) Efficacy of the varnish on sound flattened dentin and on demineralised roots.
Results of a separate in sira study show that the varnish investigated has poten-
tial to reduce caries also in already partially demineralised roots. This is impor-
tant because it is difficult to estimate clinically whether a root is still sound or
already somewhat demineralised.
The varnish was found to be less effective on flattened dentin than on intact
roots.
3) The mechanism of varnish action. The varnish releases the antimicrobials
firstly into the saliva, into the pellicle and into the hard tissues. Because the
varnish adheres to the tissues for considerable periods (at least 2 days), the last
point mentioned is important. The penetration depth of chlorhexidine into den-
tin is estimated in this work to be ± 100-300 μm. The relative importance of the
antimicrobials released from the tissue, pellicle and saliva in due time is presently
unknown. It is assumed, however, that the presence of chlorhexidine in the
dentin and its subsequent release from this tissue are important mechanistic
aspects in root caries prevention.
4) Clinical use of antimicrobial-releasing varnishes. Some recommended steps
for clinical use of the varnish are listed.
5) Future varnish developments. Some varnish improving possibilities are con-
sidered.

From this thesis it can be concluded that antimicrobial releasing varnishes effec-
tively reduce root surface caries as well as enamel caries in situ. This material will
most likely play an important role in professional caries prevention in the future.

and enamel B_{max} values were ± 1.3 mg and 0.3 mg per gram material,
respectively, equivalent to about 0.4 and 0.03 mg m^{-2}.

It is concluded that:
1) chlorhexidine binds mainly to the organic matrix in dentin and in enamel.
2) chlorhexidine can diffuse into the hard tissues.

In chapter 8, the general discussion some important aspects of varnish application
are discussed:

1) Differences between in situ and in vivo experiments. The possible differences
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Presently, there are no reasons to assume that the efficacy of the plaque situation
under consideration is very different in in situ and in in vivo conditions.
Recently, the varnish (Cervitec*) effect under normal in vivo conditions was
reported and showed to be 3-4 months.
2) Efficacy of the varnish on sound flattened dentin and on demineralised roots.
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