IN VIVO REMINERALIZATION OF ARTIFICIAL CARIOUS LESIONS USING CALCIFYING SOLUTION AND FLUORIDE SOLUTION

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Abstrak


Abstract

The remineralization potential of fluoride solution and calcifying solution was studied using an in situ model. Matched enamel discs of artificially demineralized human enamel were attached to an acrylic mandibular removable appliance of 6 adult volunteers who rinsed their mouth with a solution containing either 50 ppm F-, 200 ppm F- in amine fluoride, calcifying solution (formula BR21) or placebo as a control. The volunteers were asked to rinse 3 times a day for 3 minutes with 15 ml of the solution for 6 days (18 times). On the 7th day enamel discs were taken out from the partial denture. Three kinds of measurements were done: enamel permeability testing, depth lesion testing and fluoride retention testing. New demineralized enamel discs were attached to the partial dentures and the volunteers were asked to start rinsing with other solutions using the same protocols. The purpose of this study was to answer the question why the calcifying solutions were no more used as a remineralizing solution. The data was analyzed using SPSS/PC for two factors Anova and one way Anova for enamel permeability and Kruskal Wallis for analyzing enamel depth lesion and fluoride retention. There was no significant difference after rinsing with
In Vivo Remineralization

calcifying solution and placebo in enamel permeability, depth lesions and fluoride retention when compared to fluoride solutions. To get a better result in remineralizing carious teeth fluoride contents in solution must be higher than 50 ppm F-.

Introduction

Caries is a process of dissolution of the mineral phase of enamel by acids formed in the plaque. In the oral environment, mineral loss occurs several times a day when microorganisms, food substrates and tooth material interact. An enamel surface, partially covered by dental plaque, may come under acid attack if nutrients suitable for the plaque metabolism present.

Depending of the type and duration of the acid attack, the presence of inhibitors (e.g. fluoride) as well as the acid resistance of enamels, mineral will dissolve and outward diffusion of the mineral component calcium and phosphate will take place. In general practice the first detectable clinical sign of an early caries lesion is “The White Spot”. No cavitation is initially evident but the enamel is already softer than the surrounding sound enamels cross section through such a spot (“the lesion”) reveals a subsurface defect with a relatively intact surface layer.

Calcium and phosphate is lost from the tooth during the demineralization process and may be either precipitated somewhere else in the tooth, or lost to the mouth via the plaque and saliva. In the remineralization process, calcium and phosphate diffuses into the tooth from saliva and/or plaque fluid and precipitates as new material inside the early carious lesion. Remineralization is an enrichment of the partially demineralized tissue via formation of redeposit mineral. The redeposit mineral is normal crystal growth on existing partially demineralized crystal but could also be new crystal formation.

Remineralization of early caries lesions has been described as early as Head1, Backer2, and Dirks3 convincingly proved that white spots, detected clinically and bite wing examination, could visibly disappear as a result of repair mechanism in saliva combined with good oral hygiene and the presence of fluoride. Extensive research, both in vitro and in vivo on remineralization phenomena has been carried out in the last two decades; but the large majority of the reports pertain to in vitro work4,5,6,7,8,9, suggested that a natural biological remineralizing process exists in the mouth and that as an understanding of the complex physical and chemical reactions improve. The could be a real hope that truly effective prophylaxis against dental caries might be evolved.

Featherstone5 modified the formulation of remineralizing solutions that could possibly be applied clinically (formula BR21) which showed most promise. They added ions such as Mg, Zn, Sr etc. for faster diffusion and precipitation process. Since 1990 there no research the use of calcifying solution, just disappeared. Most of the study has been done using fluoride solutions.

The purpose of this study is to answer the questions why the calcifying solutions were not used anymore as remineralizing solutions.
Materials and Methods

In vivo, remineralization was carried out using an artificial carious human enamel disc that was attached to the lingual flange of acrylic mandibular removable appliance and worm by the volunteers for the remineralization process.

In Vitro Artificial Carious Lesions

The initially intact sound human enamel of upper first premolars teeth was extracted as part of their orthodontics treatment, were demineralized* in vitro using 6% hydroxy ethyl cellulose in 0.1M lactic acid at pH 4.5 ("Gray Formula", Gray \(^{10}\), with addition of 0.1% Thymol \(^{11}\).

First, a “window”, was prepared by placing and annullar adhesive tape (circular area 5.4 mm\(^2\)) on the buccal surface to demarcate the biopsy site. The annullar adhesive tape was positioned carefully to ensure good marginal adaptation on an experimental tooth and at the same distance (1.5 mm) from the incisal edge. A thin layer of nail lacquer was painted around the tooth. Three, was the parafilm removed before the nail lacquer hardened completely and cleaned with “cleaning solution” \(^{12}\). A hole was made on the apex of each tooth and each tooth was suspended with a nylon rope in a small polystyrene bottle containing 40 ml of a “demineralizing solution” in as incubator at 370° C. for 9 days to produce a lesion depth of 127.7um. \(^{11}\). The artificial carious lesion was cut 1mm. From the outer peripheral border to a depth of 2-mm using a water-cooled diamond wheel. This part was called “enamel disc”. Finally, two enamel discs were mounted bilaterally on the lingual flange of an acrylic mandibular appliance on the first permanent molar region.

Participants

Six human volunteers (ranging in age 23-37 years) wore the mandibular appliance with the two enamel discs put on it, day and night except during cleaning for 6 days. The participants were instructed to clean the appliance after every meal 3 times a day with tap water and smooth brush without tooth paste, and after that to rinse with 15 ml of “Remineralizing solution” for 3 minutes, 3 times a day (18 times for every remineralizing solution). 30 minutes after rinsing the participant were not allowed to drink or eat. On the 7\(^{th}\) day the appliances were collected and enamel discs were removed from the appliance for measurements. New enamel discs were put on the appliance and the participants were instructed to rinse with another remineralizing solution using the same protocols.

Outline of Experiments

The remineralizing solutions used:
1. Calcifying solution (formula BR 21), Featherstone\(^{5}\)
2. 50 ppm amine fluoride 242 (= Hexa de cylaminehydro fluoride = Cetyla minehydrofluoride) (Merck).
3. 200 ppm amine fluoride 242 (=AmF)
4. Unionized water as placebo,
5. Added peppermint oil, saccharin and methyl p-hydrobenzoate were to every solution.

On the 7\(^{th}\) day, enamel discs were removed from the appliance and mounted on a block of wax for following measurements.
In Vivo Remineralization

1. Permeability test ("Back Diffusion Technique") by Bakhos12 that was evaluated for its accuracy by Amdur13 and Tavares14.
2. Solubility / depth lesion measurements.
3. Fluoride acquisition measurements.

Solubility/depth measurements and fluoride acquisition measurements were done at the same time using "Biopsy Technique"15,16,17. The data was analyzed using SPSS/PC for two factors Anova and one way Anova for enamel permeability; Kruskal Wallis was used for analyzing enamel depth lesion and fluoride retention ( \( \chi = 0.001 \)).

Results

Permeability measurements

Permeability measurements show the amount of minerals deposited on the enamel surface. Figure 1 shows the results from the permeability measurements after remineralization on artificial carious enamel discs with placebo and formula BR21; the mean was 23.89 \( \pm \) 2.50 and 21.25 \( \pm \) 3.03 ppm, respectively. Statistical analysis revealed no differences between the two. After remineralization with AmF50 and AmF200, the result from permeability measurements were 17.17 \( \pm \) 4.07 and 2.53 \( \pm \) 1.98 ppm, respectively.

Depth Lesion Measurements

Depth lesion measurements are defined as the amount of minerals (Ca) extracted from the biopsy area and is determined by atomic absorption spectrometer. The depth of etch is calculated by the following formula:

\[
\text{Depth of etches (\mu m)} = \frac{\text{Weight of dissolved enamel}}{\text{Density of enamel \times Biopsy surface area}}
\]

Figure 2 shows that the differences of depth measurements of the artificial carious lesion after remineralization with placebo and Formula BR21 is significant (19.57 \( \pm \) 4.199 and 18.91 \( \pm \) 1.55). After remineralization with AmF50 and AmF200, the corresponding depth lesion measurement result is significant decrease in depth (15.40 \( \pm \) 1.61 and 12.61 \( \pm \) 0.78). After remineralization with AmF50 and AmF200 depth lesion measurement results are also lower than those of normal enamel (17.63 \( \pm \) 1.84).

Fluoride Acquisition Measurements

The amount of fluoride acquired by the enamel after remineralization was obtained by extracting the fluoride ions from the biopsy area and measured by using a combination fluoride electrode (Orion Res.).

Figure 3 shows the significant differences between fluoride acquisition measurements after remineralization with placebo, formula BR21, AmF50 and AmF200 on artificial carious lesion as 1660.00 \( \pm \) 185.29; 1148.33 \( \pm \) 84.94; 2337.50 \( \pm \) 106.87; 2810.00 \( \pm \) 69.92, respectively. The results show that the acquired fluoride values strongly increase after remineralization with AmF50 and AmF200. After remineralization with Formula BR21, fluoride acquisition measurement was lower than those of normal enamel (1225 \( \pm \) 35.79) 30.
Fig. 1 The mean value of permeability measurement after remineralization with placebo, BR-21, AmF 50 ppm, F− 200 ppm compare to normal enamel and initial caries.

Fig. 2 The mean value of depth lesion measurement after remineralization with placebo, BR-21, AmF 50 ppm, F− 200 ppm compare to normal enamel.

Fig. 3 The mean value of fluoride acquisition measurement after remineralization with placebo, BR-21, AmF 50 ppm, F− 200 ppm compare to normal enamel.
Discussion

Remineralization is the process by which-dissolved crystals are induced to grow by precipitation of mineral ions from solution, so that the process of mineral loss is to some extent reversed. One can see the remineralization potential of the four remineralizing solutions by comparing it to the normal enamel and back to artificial carious lesion (before remineralization started).

Figure 1 shows that artificial carious enamel with the result from (permeability measurements of 57.98 ± 7.60 ppm) can be remineralized almost completely with AmF200 (2.34 ± 1.98) compared to normal enamel (1.46 ± 0.74) 30.

After remineralization with placebo, artificial carious lesions showed a decrease in permeability measurements of enamel from 57.98 ± 7.90 to 23.09 ± 2.51 ppm which indicates the remineralization potential of saliva in depositing minerals.

According to Shannon 18 the normal level of fluoride in human saliva is too low (0.02 ppm). Featherstone 15 suggests that the basal level of fluoride in human saliva is 0.005 - 0.026 ppm and fluoride concentration in dental plaque within 0 - 60 ppm 20. Remineralization with placebo shows, that a remineralization potential of saliva and formula BR21 (containing 1 ppm F-) the permeability measurement was decreased. This means that the depth lesion measurement values after remineralization with placebo and formula BR21 are higher than those of normal enamel. Fluoride acquisition after remineralization with placebo is higher than in a normal enamel, but after remineralization with formula BR21 the result from fluoride acquisition measurements was indicated lower than those of normal enamel.

The in vivo study are agreement with the findings of Caslavksa 21, which stated that the fluoride diffusion is faster through enamel than for the double charged calcium and phosphate ions.

Nelson 22 and Wong 23 reported that incorporated fluoride at the enamel (1000 ppm) is in effective and that much higher levels, approaching those in fluoroappetite (3000 ppm 24) are required to reduce dissolution rate significantly.

After remineralization with formula BR21 the fluoride acquisition is lower than after remineralization with placebo, the result of permeability measurement are lower and not significant, the depth lesion measurements is lower than those of placebo. It is contrary to the reported of Featherstone, that solution containing calcium and phosphate at comparable levels of whole saliva is more effective remineralizing agent than saliva itself 5.

However, in as much as the remineralization does not ordinarily proceed beyond the geometric surface of tooth, it appears that the tooth saliva interface process unique properties such as the presence of enamel pellicles and salivary macromolecules that actually inhibit the formation of basic calcium phosphates.

The depth lesion measurements after remineralization with formula BR21, and placebo also show no statistically significant difference. This indicates that the depth lesion measurements are higher than normal enamel (17.634 ± 1.839) 30, but after remineralization with AmF50 and AmF200 the results of was depth lesion measurements are lower than for normal enamel. This means that
enamel becomes harder and more
difficult to dissolve by acids.

With formula BR21 containing 1
ppm F\textsuperscript{-}, the results of depth lesion
measurements show higher values than
for normal enamel. This result support
the previous evidence that 1 ppm F\textsuperscript{-} is
not enough to remineralized enamel, at
least about 50 ppm F\textsuperscript{-} is needed.

In this study, after remineralization
with AmF50, the result is not as good as
with AmF200, permeability
measurement results almost come to
normal. The depth lesion measurements
show lower than normal enamel and
fluoride acquisition is higher (2810 \( \pm 
69.93 \)), almost reaching 3000 ppm.
Jenkins\textsuperscript{24} stated that if fluoride content
of enamel reaches 3000 ppm, the
protection ability of enamel is maximal
for two reasons:

1. That concentration is adequate
enough to change the outer layer of
crystal appetite to Fluor appetite that
protects the inner layer crystal. The
inner layer crystal is can be so easily
substituted as the outer layer.

2. When fluoride is incorporated into
the appetite crystal lattice, F\textsuperscript{-}
replaces OH\textsuperscript{+} ions.

Fluoroappetite (FA), Ca (PO\textsubscript{4}) 3F, in
which all the hydroxyls are replaced, is
more stable than HA, because of
stronger bonding to adjacent calcium
ions, and hence a more compact crystal
structure. FA also has a significantly
lower solubility product than HA\textsuperscript{25}.

Budipramana\textsuperscript{2} compares in a
previous study, the efficacy of various
amine fluoride concentrations, AmF50,
AmF100, AmF150, AmF200 and
AmF250. Those showed that after
remineralizing with AmF200 gave the
lowest permeability and depth lesion
results and the highest fluoride
acquisition. This had led to the view that
it is better to have quite low levels of
fluoride incorporation into appetite (as
low as 200 ppm) to reduce dissolution
rate in significantly.

A number of experiments have
revealed that when the driving force for
remineralization is too high (excessive
super saturation too high a fluoride
concentration), rapid crystal growth may
block the surface layer pores, so that the
underlying lesion becomes arrested
rather than remineralized\textsuperscript{9,27,28,29}.

Both saliva and plaque fluid is
supersaturated at neutral pH with respect
to hydroxyappetite and can thus support
remineralization. At the pH and super
saturation level found in “Resting plaque
fluid”, appetite crystal growth proceeds
by way of formation of intermediate
solids, especially octa calcium phosphate
(OCP) which then hydrolysis to hydroxy
appetite. In general, fluoride stimulates
appetite crystal growth. The hydrolysis
of OCP is accelerated and the product of
crystal growth is FA or FHA rather than
hydroxyappetite. Thus, it is generally
considered desirable to avoid excessive
rates of remineralization and to enhance
the effectiveness of the remineralizing
capacities of the oral fluids by making
fluoride continuously available at
suitably elevated but still quite low
concentrations. It seem reasonable to
suggest that for better results in
remineralizing carious teeth fluoride
content in solution should be higher than
50 ppm but not higher than 200 ppm F\textsuperscript{-}.

Conclusion

The study has shown that after
remineralization with a calcifying
solution (Formula BR21) appears to
make no difference to potential
remineralization of saliva itself. It also shows the enhancement potential of fluorides in remineralizing artificial carious teeth. After remineralization with AmF 200 ppm F- on artificial carious enamel for 6 days, the permeability of the enamel approaches that of normal teeth, the depth lesion results are lower and fluoride acquisition are higher than normal enamel. We conclude from this study that AmF 200 ppm F- solution gives a good effect on remineralization of artificial carious lesion and 1 ppm F- in calcifying solution has no effect in remineralized artificial carious lesion.

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