Original Paper

Caries Research

Caries Res 2010;44:380–389
DOI: 10.1159/000318224

Received: March 10, 2010
Accepted after revision: June 19, 2010
Published online: August 10, 2010

Formation of Caries-Like Lesions in vitro on the Root Surfaces of Human Teeth in Solutions Simulating Plaque Fluid

R.P. Shellis

Department of Oral and Dental Science, University of Bristol, Bristol, UK; Department of Preventive, Restorative and Pediatric Dentistry, University of Bern, Bern, Switzerland

Key Words
Caries · Dentine · In vitro · Solubility

Abstract
Lesion formation on root surfaces of human posterior teeth was studied in acetate/lactate buffers with a background electrolyte composition based on plaque fluid analyses. Lesion depth after 28 days at 37°C was measured in relation to: the presence or absence of cementum; the concentration of undissociated buffer; the presence or absence of magnesium ions at plaque fluid concentration. Each factor was evaluated at several values of –log(ion activity product for hydroxyapatite): pI_{HA}. Solutions were formulated to minimize variation in pH, which varied by ≤0.03 for a given comparison (individual pI_{HA}) and by 0.42–0.82 over the range of pI_{HA} within experiments. Lesions on surfaces from which cementum had been ground were significantly deeper than on intact surfaces, but this is considered to be due to subsurface mechanical damage and not to a solubility difference. Neither the concentration of undissociated buffer nor the presence of magnesium ions significantly affected lesion depth. Lesion depth was strongly influenced by the correlated variations in pI_{HA} and pH. At pI_{HA} 54 and 55, only extremely shallow lesions formed. From pI_{HA} 56, lesion depth increased with increasing pI_{HA}. The results confirm that the solubility of the mineral of root tissues is higher than that of hydroxyapatite, but indicate that it is probably lower than suggested by Hoppenbrouwers et al. [Arch Oral Biol 1987;32:319–322]. For calcium concentrations of 3–12 mM, the critical pH for root tissue mineral was calculated as 5.22–5.66 assuming solubility equivalent to pI_{HA} 54 and 5.08–5.51 assuming pI_{HA} 55.

Copyright © 2010 S. Karger AG, Basel

In contrast to the large number of in vitro studies aimed at defining the factors controlling the rate of formation of caries-like lesions in enamel, relatively few such studies have been published on lesion formation in root-surface tissues [Hoppenbrouwers et al., 1986, 1987, 1988; Arends et al., 1987; Featherstone et al., 1987; ten Cate et al., 1998]. Moreover, the experimental conditions used have not resembled those in vivo very closely. The aim of the present study was to examine the effects of some relevant factors on lesion formation on root surfaces, using demineralizing solutions based on analyses of plaque fluid. The factors identified for study were the degree of saturation with respect to hydroxyapatite, the substrate, the concentration of undissociated acid in the demineralizing solution and the presence of magnesium.

KARGER

Fax +41 61 306 12 34
E-Mail karger@karger.ch
www.karger.com

© 2010 S. Karger AG, Basel
0008–6568/10/0444–0380$26.00/0

Accessible online at:
www.karger.com/cre

Dr. R.P. Shellis
Department of Oral and Dental Science, University of Bristol
Dental School, Lower Maudlin Street
Bristol BS1 2LY (UK)
Tel. +44 117 342 4328, Fax +44 117 342 4778, E-Mail r.p.shellis@bristol.ac.uk

source: https://doi.org/10.48350/435 | downloaded: 10.4.2024

Downloaded from http://karger.com/cre/article-pdf/44/4/380/2499302/000318224.pdf by UniversitÃ¤tsbibliothek Bern user on 25 May 2023

A solubility product for dentine mineral has not been established. Although Paschalis et al. [1996] considered that dentine mineral behaves as a mixture of hydroxyapatite-like and octacalcium-phosphate-like phases, it is simplest to define solubility in terms of the ion activity product for hydroxyapatite [I_{HA}: $(Ca^{2+})^5(PO_4^{3-})^3(OH^-)$, where parentheses indicate ion activities in moles per litre], expressed for convenience as the negative logarithm, pI_{HA}. By using a range of pI_{HA}, lesion formation in solutions under- or super-saturated with respect to hydroxyapatite can be studied. Hoppenbrouwers et al. [1986, 1987] found that root tissues demineralize in solutions that are actually supersaturated with respect to hydroxyapatite. They observed a linear relationship between the rate of mineral loss and pI_{HA}, and estimated that the apparent solubility (i.e. the pI_{HA} at which lesions would cease to form) was pI_{HA} 52.65. Similar estimates (52.58 and 52.0) for the solubility of powdered dentine were made by Arends et al. [1987] and Paschalis et al. [1996] from equilibrations with solutions at near-neutral pH. In a previous study [Shellis, 1996], the solubility of coronal dentine seemed to be lower than this, since only very slight demineralization occurred at pI_{HA} 55. Further work thus seemed necessary.

McIntyre et al. [2000b] found that, under similar conditions, deeper lesions formed in root surfaces from which the cementum had been removed than in intact root surfaces. Since this might imply a solubility difference between dentine and cementum, studies at a range of pI_{HA} seemed to be potentially useful.

It is recognized that lesion formation on root surfaces is, as in enamel, a diffusion-controlled process [Arends et al., 1987; Featherstone et al., 1987]. Thus, any mechanism which might improve the transport of reactants (especially H^+) and products between the site of demineralization and the external bathing solution could enhance the rate of lesion formation. In the progress of caries lesions in enamel, the concentration of undissociated acid (here called [HX]) is thought to be an important factor [Gray, 1961; Featherstone and Rodgers, 1981; Theuns et al., 1984]: owing to their lack of charge, undissociated acids should be able to diffuse fairly readily into the hard tissue and there act as buffers to maintain a reduced pH and hence the undersaturation of the internal fluid. Hoppenbrouwers and Driessens [1988] suggested, from a re-interpretation of the data of Featherstone and Rodgers [1981], that the increase in rate with undissociated acid concentration was actually due to decreasing inhibition by the free acid anions, which adsorbed to the crystal surfaces. Hoppenbrouwers et al. [1987] observed large differences in demineralization rate between experiments with the same total concentration of buffer but with [HX]. However, the design of the experiments in the studies cited does not permit evaluation of the separate effects of [HX], acid anion concentration and pH. Clearly, further investigation of these possible factors in lesion formation in dentine is warranted.

Magnesium is a prominent component of plaque fluid. Paschalis et al. [1996] found that this ion had a marked inhibitory influence on dissolution of powdered dentine. It was therefore considered of interest to determine whether this ion affected lesion formation.

Root-surface lesions form in contact with plaque fluid, the interstitial fluid of plaque, and it was thought important to study the variables discussed above within the constraints of what is known of the composition of plaque fluid. Lynch et al. [2006] have adopted this approach in studies of caries formation in enamel. The aim in the present study was to select a background electrolyte composition against which variables of interest could be fixed and their influence on lesion formation studied.

Materials and Methods

General Principle

Published data on plaque fluid composition [Moreno and Margolis, 1992; Margolis et al., 1993a, b] were used to formulate solutions with electrolyte compositions similar to those of plaque fluid near the pH minimum of the Stephan curve. From these data, the concentrations of Na^+, NH_4^+, Cl^- and phosphate appeared to vary only to a limited extent, so fixed concentrations of these species, based on rounded averages of all analyses of Margolis and Moreno [1992], were used. The total concentration of organic acids was set at 80 mM on the basis of several reports [Edgar et al., 1986; Higham and Edgar, 1989; Margolis and Moreno, 1992]. The reported concentration of calcium at the pH minimum of the Stephan curve varies widely, from about 3 to about 14 mM [Margolis and Moreno, 1992; Margolis et al., 1993a, b; Vogel et al., 1998], although most values lie in the range 3–7 mM. Therefore, calcium concentrations of 3–6 mM were used.

For each treatment, a series of solutions with a range of pI_{HA}, but with constant [HX], was required. To achieve this, the number of degrees of freedom was increased by using two organic acids with different pK_a – consisting of lactic and acetic acids – to create a mixed-acid buffer. By an iterative procedure, using an ion speciation program [Shellis, 1988], the concentrations of the two acids (to the nearest 1 mM) and the pH were varied until the required [HX] and pI_{HA} were obtained. Table 1, which gives an example of such a series of solutions, shows that as pI_{HA} increased, the proportion of lactic acid increased and the pH fell slightly. The total concentration of the free anions of the two acids remained almost constant. The electroneutrality imbalance, $U_{\pm}$, is calculated from the sum of the products of concentration and charge of all the ions in solution. Here it is negative, indicating an apparent excess of

Downloaded from http://karger.com/cre/article-pdf/44/4/380/2499302/000318224.pdf by UniversitÃ¤tsbibliothek Bern user on 25 May 2023

Table 1. Example of a series of solutions with varying pI_{HA}: sums of concentrations of free acetate and lactate anions are given as well as [HX]

[Acetate], mM	[Lactate], mM	pH	pI_{HA}	$[HX]_{total}$, mM[1]	[Acetate$^-$] + [Lactate$^-$], mM	$U_{\pm}$, mM
47	33	5.26	55.03	10.0	68.0	–18.5
33	47	5.12	56.06	10.0	67.8	–18.4
23	57	4.99	57.01	10.1	67.6	–18.1
14	66	4.85	58.02	10.0	67.7	–18.1
6	74	4.71	59.02	9.8	67.8	–18.3

All solutions contained 6 mM Ca, 40 mM K, 15 mM Na, 20 mM NH_4, 15 mM phosphate and 20 mM Cl. $U_{\pm}$ = Electroneutrality imbalance (sum of the charges of all anions and cations in solution). [1] Includes the small concentration of H_3PO_4.

anions, and $-U_{\pm}$ = the KOH concentration required to achieve the given pH. Hence $-U_{\pm}$ was added to the K^+ added initially to give the total K^+ concentration (overall range 55–68 mM).and was included in calculation of ionic strength.

Solutions

Solutions were prepared from analytical grade reagents obtained from Merck-BDH (Lutterworth, Leicestershire, UK) or Aldrich (Poole, Dorset, UK). All solutions contained 15 mM NaH_2PO_4, 20 mM NH_4Cl and 40 mM KOH to give a background electrolyte composition of 15 mM Na, 20 mM NH_4, 20 mM Cl, 15 mM phosphate. Concentrated stock solutions of these salts were added to about 1.8 litres of water, together with appropriate volumes of 2 M acetic and lactic acids to give a total organic acid concentration of 80 mM. Weighed quantities of $CaCO_3$ and, in 2 experiments, hydrated magnesium carbonate, were added and allowed to dissolve before adjusting the pH with KOH solution and bringing the volume to 2 litres: sufficient for 1 experiment. Thymol crystals were added to inhibit microbial growth and the solutions stored in an incubator at 37°C.

Preparation of Teeth

Caries-free permanent premolars and molars were selected from a collection of teeth stored in 70% ethanol. It was not known whether the cervical surfaces had been exposed to the oral environment in vivo. The root surfaces were cleaned using a pumice slurry and a slowly-rotating brush, the teeth washed in tap water and the crowns cut off at the enamel-cementum junction with a diamond wheel. Each root was sectioned longitudinally into 3 or 4 specimens. Before each experiment, each root specimen was attached to a length of stainless-steel wire passed through a small hole drilled near the apex. The specimens were blotted dry and experimental windows were defined by fixing strips of adhesive tape, 3 × 0.5 mm, transversely on the root surface in the cervical region. Nail varnish was then applied to all surfaces of the specimens. After the varnish was dry, windows were opened by carefully cutting along the edges of the adhesive strips with a scalpel and removing the strips with forceps.

Experiments

In all experiments, there were 5 root specimens in each treatment group. There was no formal power calculation but it was considered that, since the adjusted means were tested by paired comparisons on the combined data from 3 or more treatment groups, the total numbers would be adequate.

Experiments 2–4 involved sequential exposure of windows to solutions varying in a factor of interest. Two or three windows were placed on each specimen. Using random number sequences, one window was opened and exposed to the first solution. This window was then varnished and the second window opened and exposed to the second solution. This procedure was repeated for a third time in experiment 2. The exposure of windows in random order was intended to eliminate any influence of position. In all experiments, root specimens were exposed in an incubator at 37°C to 8 changes of 200 ml of test solution. The compositions of the test solutions are summarized in table 2. Each group of specimens was suspended by the attached stainless-steel wires from a strip of autoclave tape spanning the mouth of a screw-cap polypropylene bottle (Nalgene). All exposures lasted for 28 days and the solutions were changed twice per week. The pH was checked and adjusted before each change of solution. The choice of experimental duration, solution volume and exchange frequency were based on earlier work [Shellis, 1996] using similar solutions. In the early experiments the pH of used solutions was checked, and found to vary by no more than 0.02 units from the set pH. At the end of each experiment, the specimens were removed, washed in tap water and stored in 70% ethanol in separate vials after removing the wires.

Experiment 1. The aim was to evaluate the effect of cementum removal. The experiment was performed using 2 calcium concentrations. Using a slowly rotating diamond disc lubricated with water, about 0.5 mm was carefully ground from the surface in the cervical region of each of 100 root specimens to create a strip, about 1 mm wide, of cementum-free surface. Windows were created on both the ground surface and on the intact surface immediately apical to it: both were left open throughout the experiments. Ten groups of specimens were exposed to Mg-free solutions with [HX] = 10 mM, containing either 3 or 6 mM Ca. In the 3 mM Ca experiment, pI_{HA} ranged from 55 to 59, and in the 6 mM experiment from 54 to 59.

Experiment 2. The aim was to evaluate the effect of [HX] on lesion formation. The experiment utilised 3 sets of solutions, each with pI_{HA} varying from 56 to 60, containing 5 mM Ca and with [HX] being 7, 10 or 15 mM. In each experiment, root specimens were prepared with 3 windows, and were exposed to the 3 solutions. One window on each specimen was exposed in random order to the solutions with [HX] = 7, 10 and 15 mM.

Experiment 3. The aim was to evaluate the effect of magnesium on lesion formation at 2 values of [HX]. Two sets of solutions, with pI_{HA} 56, 57 or 59, were prepared. Each contained 3 mM Ca, [HX] was 6 or 10 mM and the magnesium concentration was 0 or 2.5 mM. The chosen magnesium concentration is somewhat above the mid-range of the values reported by Margolis and Moreno [1992] and Margolis et al. [1993a, b]. Each root specimen had 2 windows, which in random order were exposed first to the magnesium-free solution and then to the corresponding magnesium-containing solution.

Experiment 4. This experiment was designed to test the effect of magnesium. Six solutions, with pI_{HA} 56, 58 or 60, 5 mM Ca, 10 mM [HX] and 0 or 2.5 mM [Mg] were used. Three groups of

Downloaded from http://karger.com/cre/article-pdf/44/4/380/2499302/000318224.pdf by UniversitÃ¤tsbibliothek Bern user on 25 May 2023

Table 2. Summary of solution compositions in experiments 1–3

Experiment	pH	[Ca], mM	[Mg], mM	pI_{HA}	$[HX]_{total}$, mM	[Acetate] + [Lactate], mM
1	4.92–5.46	3	0	55.01–59.03	10.0 ± 0.1	68.8–69.1
	4.71–5.38	6	0	54.13–59.02	10.0 ± 0.1	67.9–68.2
2	4.77–5.19	5	0	56.07–59.06	7.0 ± 0.1	70.9–71.1
	4.76–5.18	5	0	56.02–59.06	10.0 ± 0.1	68.0–68.3
	4.74–5.15	5	0	56.05–59.08	15.0 ± 0.1	62.2–63.5
3	4.93–5.35	3	0	56.02–59.03	6.1 ± 0.1	72.7–72.8
	4.64–5.46	3	0	54.97–60.98	10.0 ± 0.1	68.7–69.1
	4.93–5.36	3	2.5	55.98–59.05	6.0 ± 0.1	72.0–72.1
	4.64–5.46	3	2.5	55.00–61.00	10.0 ± 0.1	68.0–68.5
4	4.62–5.18	5	0	56.02–60.06	10.1 ± 0.1	68.0–68.3
	4.62–5.18	5	2.5	56.02–60.06	10.0 ± 0.1	67.2–67.4

All solutions contained 40 mM K, 15 mM Na, 20 mM NH_4, 20 mM Cl, 15 mM phosphate and 80 mM organic acids (lactic + acetic).

root specimens, each with 2 windows, were exposed first to the magnesium-free and magnesium-containing solutions as in experiment 3.

Evaluation of Lesions

Longitudinal sections of the specimens, passing through the centre of the lesions, were cut using a slowly-rotating water-lubricated annular diamond-edged saw (Microslice 2: Malvern Instruments, Malvern, UK). Sections were polished to approximately 80 μm using a slurry of 1200 mesh SiC powder in water.

Lesions were quantified by measurement of depth, using a polarizing microscope fitted with an eyepiece crosswire with one calibrated axis. Some root-surface lesions showed a clear boundary in ordinary light [fig. 6, 7 in Shellis, 1994], which marks an abrupt change in the refractive index between sound and demineralised dentine. However, this boundary is not a suitable marker for the measurements of lesion depth. First, only some lesions show such a distinct boundary [fig. 6 in Shellis, 1994]. Secondly, when lesions were mounted in quinoline and examined in polarized light, they appeared as a negatively birefringent zone separated from the underlying positively birefringent sound dentine by a pseudo-isotropic zone, as described for artificial lesions formed in undersaturated buffer by McIntyre et al. [2000a], and the latter extended somewhat beyond the visible boundary when this was present. Thus, sections were dehydrated in ethanol and mounted in quinoline, and the boundary between the isotropic tissue and the sound dentine was taken as the inner boundary. In most lesions, which showed no sign of shrinkage, the outer lesion surface was defined by the root surface. Some of the more advanced lesions showed surface shrinkage and in measuring these lesions the plain axis of the crosswire was placed at the intact surfaces bordering the lesion and measurements taken from this line [Shellis, 1994]. For each lesion, 3–5 measurements were made in the middle third and a mean calculated.

To assess the effectiveness of the method of compensating for shrinkage, 25 lesions with an optically visible inner boundary were identified. The depth was measured to this boundary in ethanol: first in relation to the lesion surface and also, when shrinkage was visible, using the cross-wire as a reference. Shrinkage was measured directly as the maximum distance between the lesion surface and the cross-wire positioned on the root surface on either side of the lesion. The sections were then re-measured after thorough re-hydration in water, using the lesion surface as a reference.

Statistical Analysis

Agreement between lesion depth in water with lesion depth in ethanol measured using the cross-wire method was assessed using a Bland-Altman plot [Bland and Altman, 1999].

Lesion depth data were tested for normality by the Shapiro-Wilks test and for homogeneity of variance between groups by Levene's test. The experiments were analysed using a general linear repeated-measures model, with 'state' (ground/intact) (experiment 1), '[HX]' (experiment 2) and 'magnesium' (presence/absence) (experiment 3 and 4) as within-group factors. Because, within each experiment, variations in pI_{HA} were accompanied by variations in pH, the various combinations of pI_{HA} and pH ('pI_{HA}/pH') were used as a between-group factor. For experiment 3, '[HX]' was an additional between-group factor. In experiment 1, because there was a consistent difference in pH between solutions with corresponding pI_{HA} values but different calcium concentrations, separate analyses of the differences between ground and intact root surfaces were run for the 2 sets of solutions. In all analyses an α value of 0.05 was used.

Statistical calculations were performed using SPSS v.16.0 (SPSS UK Ltd, Woking, Surrey, UK) and XLStat software v. 7.5 (Addinsoft, New York, N.Y., USA).

Results

Of the 25 lesions used to compare lesion depths measured in ethanol using the cross-wire method and in water, 4 were excluded because they showed shrinkage in

Downloaded from http://karger.com/cre/article-pdf/44/4/380/2499302/000318224.pdf by UniversitÃ¤tsbibliothek Bern user on 25 May 2023

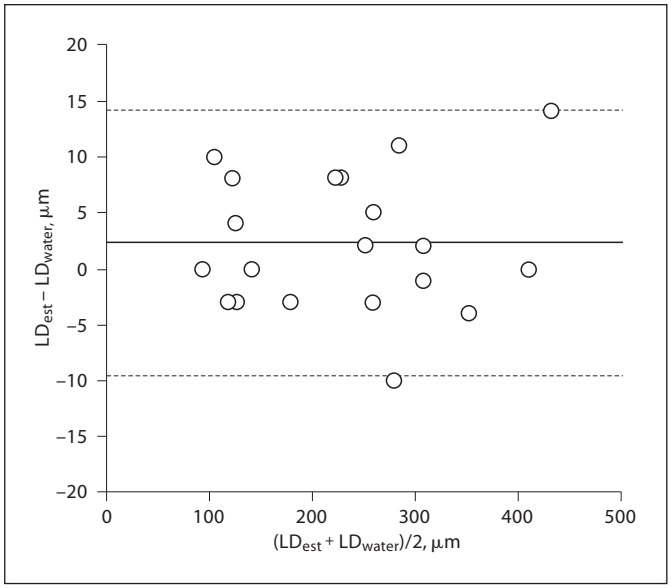

Fig. 1. Bland-Altman plot to show the level of agreement between lesion depth measured on hydrated sections (LD_{water}) and lesion depth measured on sections dehydrated in ethanol, using the cross-wire method (LD_{est}).

water, so did not provide a valid basis for comparison. The mean depth in water of the remaining lesions was 233 μm (range 50–426 μm). The mean shrinkage in ethanol was 9.3 ± 8.6 (SD) μm (range 0–25 μm). A Bland-Altman plot (fig. 1) indicated a mean difference between measurements in ethanol using the cross-wire method and measurements in water of +2.3 μm with 95% confidence limits of –9.7 to +14.2 μm.

Table 2 shows that, within each experiment, pH varied by 0.42–0.82 units over the range of pI_{HA} used. In experiment 1, the pH values of solutions containing 6 mM Ca was lower by 0.2–0.21 units than those with 3 mM, at corresponding pI_{HA}. For each pI_{HA}/pH combination included in the statistical analyses, variations between different levels of the within-groups factors were very small: SD = 0–0.04 for pI_{HA} and 0–0.02 for pH (table 3).

All solutions were undersaturated with respect to octacalcium phosphate and brushite. In experiments 3 and 4, the magnesium-containing solutions with $pI_{HA} \leq 58.6$ had pI values for magnesium whitlockite of 99.44–103.21 and so would be supersaturated with respect to this solid according to the data of Verbeeck and de Bruyne [1989] and Shellis et al. [1997].

Except in experiment 1, lesions usually formed on surfaces covered with acellular cementum but sometimes on surfaces covered with cellular cementum. In experiment 1 (6 mM Ca), extremely shallow lesions (10–15 μm deep), showing reversed sign of birefringence, were observed on both ground and intact surfaces exposed to pI_{HA} 54.13 and 55.01. In experiment 3, similar lesions were observed on specimens exposed to magnesium-containing solutions with pI_{HA} 55, but on surfaces exposed to the corresponding magnesium-free solutions lesions were much thinner (ca. 5 μm) and often appeared as zones of weakened, rather than reversed, birefringence. The bodies of all lesions formed at $pI_{HA} \geq 56$ showed reversed sign of birefringence in quinoline. In experiment 2, the depths of some lesions formed at pI_{HA} 56 could not be measured accurately because, while the sites of the lesions were identifiable because of the reversed sign of birefringence of the cementum, the inner boundary of the lesion fell within the hyaline layer of the dentine, which is isotropic, like the inner margin of the lesion. Consequently, the pI_{HA} 56 lesions were excluded from statistical analysis of this experiment.

In every experiment, between-group differences with pI_{HA}/pH were highly significant ($p < 0.001$). No significant interactions between pI_{HA}/pH and the within-group variables were observed.

Experiment 1

In all specimens, the grinding process successfully removed the acellular cementum in the cervical region. Lesions on ground surfaces were on average deeper than on intact surfaces, although some lesions showed no difference, or a small difference in favour of the intact root surfaces. The mean difference was 27 ± 37 μm in the 3-mM Ca solutions and 46 ± 54 μm in the 6-mM Ca solutions. Both differences were significant ($p = 0.009$ and < 0.001, respectively). By one-way ANOVA, the difference in lesion depth between ground and intact surfaces between the pI_{HA}/pH groups was not significant ($p = 0.454$ and 0.414 for the 3- and 6-mM Ca groups, respectively).

Experiment 2

Depths of lesions in solutions with different concentrations of undissociated acid were not significantly different ($p = 0.351$).

Experiment 3

The depths of lesions formed in the absence and presence of Mg did not differ significantly ($p = 0.404$). [HX] was not a significant between-groups factor ($p = 0.830$).

Downloaded from http://karger.com/cre/article-pdf/44/4/380/2499302/000318224.pdf by UniversitÃ¤tsbibliothek Bern user on 25 May 2023

Table 3. Lesion depths in experiments 1–4 (means ± SD)

Experiment	pI_{HA}	pH	[HX], mM	Lesion depth, μm		
				ground	intact	
1 (3 mM Ca)	56.02	5.33	9.9	89 ± 42	81 ± 43	
	57.06	5.19	10.0	216 ± 41	200 ± 27	
	58.02	5.06	9.9	301 ± 40	268 ± 21	
	59.03	4.92	10.1	395 ± 38	347 ± 50	
1 (6 mM Ca)	56.06	5.12	10.0	117 ± 19	101 ± 27	
	57.01	4.99	10.1	297 ± 62	254 ± 25	
	58.02	4.85	10.0	431 ± 49	405 ± 62	
	59.02	4.71	9.8	502 ± 76	428 ± 29	
				[HX] = 7 mM	[HX] = 10 mM	[HX] = 15 mM
2	57.05 ± 0.03	5.04 ± 0.02		135 ± 33	155 ± 60	132 ± 16
	58.06 ± 0.01	4.90 ± 0.02		331 ± 91	305 ± 81	339 ± 41
	59.06 ± 0.00	4.76 ± 0.02		341 ± 40	409 ± 59	397 ± 75
				[Mg] = 0	[Mg] = 2.5 mM	
3	56.00 ± 0.03	5.36 ± 0.01	6.1 ± 0.1	135 ± 51	130 ± 36	
	55.99 ± 0.01	5.33 ± 0.00	9.9 ± 0.0	111 ± 47	73 ± 8	
	57.01 ± 0.03	5.22 ± 0.01	6.0 ± 0.1	190 ± 36	208 ± 51	
	57.00 ± 0.04	5.20 ± 0.01	10.0 ± 0.0	211 ± 28	205 ± 30	
	59.04 ± 0.01	4.93 ± 0.00	6.0 ± 0.0	396 ± 80	423 ± 37	
	58.99 ± 0.01	4.92 ± 0.00	10.1 ± 0.1	458 ± 50	408 ± 46	
				[Mg] = 0	[Mg] = 2.5 mM	
4	56.02	5.18	10.1 ± 0.1	69 ± 7	67 ± 10	
	58.06	4.90	10.1 ± 0.1	290 ± 30	254 ± 12	
	60.06	4.62	10.1 ± 0.1	468 ± 48	426 ± 81	

Where pI_{HA}, pH or [HX] did not vary between different levels of the within-group factors, one value is given. Where there was variation, means ± SD are provided.

Experiment 4

The data for this experiment showed heterogeneous variance. This problem was solved by using a $1/\sqrt{Y}$ transformation. Depths of lesions formed in the presence or absence of magnesium were not significantly different ($p = 0.192$).

Discussion

Quinoline affects the birefringence of partly-demineralized dentine through the von Ebner phenol reaction: a reversal of birefringence caused by orientated adsorption of aromatic molecules on collagen fibres exposed by mineral loss [Schmidt and Keil, 1971]. The pseudo-isotropy seen at the advancing front of lesions represents a stage of demineralization where the positive birefringence of the matrix collagen fibres and the positive form birefringence due to orientated sub-microscopic pores are compensated by the weak intrinsic negative birefringence of the remaining mineral and the strong negative birefringence due to the phenol reaction. In previous studies the boundary between the pseudo-isotropic dentine and the sound dentine was shown to correspond closely with the limit of the lesion as seen by microradiography [Hals and Nernaes, 1971; Wefel et al., 1985].

Although the lesion depths measure the depth to which mineral loss has occurred, polarizing microscopy does not, unfortunately, provide information about the distribution of mineral within the lesion. Both the min-

Downloaded from http://karger.com/cre/article-pdf/44/4/380/2499302/000318224.pdf by UniversitÃ¤tsbibliothek Bern user on 25 May 2023

eral and organic components in collagenous hard tissues have only weak intrinsic birefringences [Ascenzi, 1950]. In water positive form birefringence, and in quinoline the negative birefringence due to the phenol reaction, are dominant, so changes in mineral concentration have very little effect on the net birefringence. In vitro lesions formed in gel systems or in fluoride-containing systems demonstrate surface layers with a higher mineral content than the underlying lesion [ten Cate et al., 1995, 1998]. However, lesions formed in fluoride-free undersaturated buffers do not have a surface layer but show a continuous increase in mineral concentration from the surface towards the advancing front of the lesion [Damen et al., 1998; ten Cate et al., 1998]. The lesions in the present study are probably of this type.

In the lesions studied here, the partly demineralized collagenous matrix was retained, whereas in vivo this is degraded by the action of bacterial, salivary and endogenous proteolytic enzymes and by physical abrasion. Because the retained demineralized layer will slow down diffusion, the rate of increase of lesion depth will probably not be linear but will decrease with time. The final depth after the fixed time of the experiments will, however, reflect the overall rate.

In lesions formed in fluoride-free buffers, the parameters of lesion depth and mineral loss seem to be correlated and both increase as pH/degree of saturation decreases [ten Cate et al., 1998]. It therefore seems reasonable to assume that, for those experiments where inhibitors of demineralization were absent, lesion depth is a reliable indicator of lesion severity. However, this assumption might not hold for lesions formed in the presence of magnesium, as this is a potential inhibitor [Paschalis et al., 1998]: this is discussed later.

Because dehydration was necessary to infiltrate the lesions with quinoline, lesion depth measurements were susceptible to shrinkage error. The method used to correct lesion depth measurements for the shrinkage was used previously by ten Cate et al. [1991]. The results in figure 1 indicate that this is a reasonably satisfactory method for estimating the correct lesion depth. The degree of shrinkage was much less than has been observed in previous studies dealing with microradiography [ten Cate et al., 1991; Ruben and Arends, 1993]. It is possible that dehydration in ethanol causes less severe shrinkage than does drying in air, as is necessary for microradiography, because the collagen fibres would still contain liquid. It is also possible that the rather narrow windows will have limited the amount of possible shrinkage.

In formulating the solutions, the main objective was to fix the undissociated acid concentration, free anion composition and pI_{HA} within narrow limits, while maintaining an otherwise constant electrolyte composition. Because of the latter condition, within the series of solutions in each experiment, there was some variation in pH with pI_{HA}. In effect, each series of solutions resembled a Stephan curve, in which decreasing saturation with respect to hydroxyapatite was correlated with falling pH. A big difference from an in vivo Stephan curve is that the total organic acid concentration was constant for experimental reasons, instead of increasing as pH fell. Paschalis et al. [1996] found that pH seemed to have a greater effect on the dissolution rate of powdered dentine than did pI_{HA}. While the variations in pI_{HA} and pH between different levels of the within-group variables were extremely small, the pH variations between different pI_{HA} values were too large to allow use of pI_{HA} as an independent variable, and the composite variable 'pI_{HA}/pH' was used instead. For the same reason calcium concentration was not used as an independent variable in experiment 1. In future work, pH variation could probably be avoided if desired by adding another degree of freedom, such as allowing the total acid concentration, or the calcium or phosphate concentration, to vary.

The present results suggest that, in contrast to enamel, the concentration of undissociated acid does not affect the rate of lesion formation in root-surface tissues. It is true that the range of values of [HX] studied was relatively narrow, but this reflects the constraints due to pH and the composition of plaque fluid. Moreover, marked effects were observed at pH 5.0 over a similar range of [HX] by Featherstone and Rodgers [1981]. The reason for this difference from enamel lesions is not clear, because in both this study and in that of Featherstone and Rodgers [1981] the outer part of the lesion consisted of a diffusion-restricting layer (demineralized dentine and partly-demineralized enamel, respectively) of quite similar thickness. It is possible that, because of the lower mineral concentration and higher permeability of root-surface tissues, even low concentrations of undissociated acid can maintain a reduced internal pH, so that increasing the buffer capacity does not affect the rate of lesion formation. Another possibility is that, because partly-saturated solutions were used, lesion formation was relatively slow, and demineralizing conditions could have been maintained by even low [HA]. Lesion formation in plain acid buffers, as in the experiments of Featherstone and Rodgers [1981], would be faster, and might depend more strongly on [HX] because of the more rapid consumption

Downloaded from http://karger.com/cre/article-pdf/44/4/380/2499302/000318224.pdf by UniversitÃ¤tsbibliothek Bern user on 25 May 2023

of H^+ at the advancing front. This would imply that [HX] might be a more important factor in root-surface lesion formation at lower pH (greater undersaturation) than those used here. However, even at a very low plaque pH, the value of [HX] would be limited. Assuming the electrolyte composition used in experiment 4 plus 2.5 mM Mg, with an extreme plaque pH of 4.0 [Imfeld, 1977] and the highest acid concentrations given by Margolis et al. [1993b] (2 mM succinate, 15 mM acetate, 10 mM propionate, 60 mM lactate), [HX] can be calculated to be 45.5 mM.

Some data from kinetics studies supports to some extent the alternative hypothesis of Hoppenbrouwers and Driessens [1988], that enamel lesion formation rate varies inversely with free acid anion concentration rather than directly with undissociated acid concentration. Inhibition by adsorbed acids and their anions has been proposed as a mechanism to explain the dissolution behaviour of hydroxyapatite and powdered enamel mineral in the presence of organic acids [Voegel et al., 1983; Budz et al., 1988]. In experiments 2 and 3, the variations in the total concentration of acetate + lactate were similar in magnitude to those in [HX], so the statistical analysis suggests that acid anion concentration, like [HX], does not significantly influence lesion progression. However, all solutions contained at least 62 mM acid anions: a concentration which the data of Voegel et al. [1983] and Budz et al. [1988] suggest would be inhibitory. It is therefore conceivable that inhibition was maximal even in the solutions with the smallest value of [HX].

Hoppenbrouwers et al. [1987] studied lesion formation in dentine in 4 series of acetate buffers with varying pI_{HA}. These series of solutions varied in pH (from 5.0–6.5) and also, because the acetate concentration was constant, in undissociated acid concentration (from 0.2 to 16 mM), and in free anion concentration. Assuming that the latter factors are not important in formation of lesions in dentine, their results seem to be consistent with those of Paschalis et al. [1996], since the variations in integrated mineral loss were much larger between different pH values than between different pI_{HA} at any given pH.

The first signs of demineralization were seen at pI_{HA} 54 and 55, and lesion depth increased with increasing pI_{HA}. Formation of lesions at pI_{HA} 56–58 confirms previous findings [Hoppenbrouwers et al., 1986, 1987; Arends et al., 1987; Paschalis et al., 1996] that much of the mineral in dentine has an elevated solubility, since these solutions were supersaturated with respect to hydroxyapatite. The cited studies provided estimates of the apparent solubility of root tissue mineral as pI_{HA} = 52.0–52.7. However, since enamel is known to have heterogeneous solubility [Patel and Brown, 1975; Shellis, 1996], it seems very likely that dentine also has a range of solubilities, so it might not be appropriate to assume a single value for solubility. The suggestion [Paschalis et al., 1996] that dentine is a mixture of apatite-like and octacalcium-phosphate-like phases is one possible model to account for variable solubility. Moreover, a value of pI_{HA} as low as this might overestimate the solubility. Since Arends et al. [1987] and Paschalis et al. [1996] equilibrated powdered samples with neutral solutions, the values of pI_{HA} they reported might reflect the solubility of only the more soluble mineral. In the present study, lesions formed in solutions with pI_{HA} of 54 or 55 could be due to loss of this soluble fraction, since they were extremely shallow and did not increase in depth between pI_{HA} 54 and 55. Some published data support the conclusion that the mineral solubility of root tissues is lower than suggested by Hoppenbrouwers et al. [1986, 1987]. In a previous study, only very slight dissolution of intertubular dentine was observed even after 30 days' exposure to solution with pI_{HA} 55 [Shellis, 1996]. ten Cate et al. [1998] found that dissolution of dentine in fluoride-free solutions occurred at pI_{HA} 56 but not at pI_{HA} 53. Hoppenbrouwers et al. [1987] estimated that the critical pH for formation of caries lesions on root surfaces would be 6.7. Aamdal-Scheie et al. [1996] considered that this could not be valid because the pH of plaque on exposed but sound root surfaces of elderly subjects was frequently lower than this value. From the foregoing, it is of interest to calculate the critical pH on the assumption that the solubility of root mineral is equivalent to pI_{HA} 54–55 rather than less than 53. Accordingly, the critical pH was calculated by an iterative procedure using the program of Shellis [1988], assuming the same concentrations of background electrolytes and inorganic phosphorus as used in this study, plus 70 mMl potassium, 2.5 mM magnesium, a partial pressure of 0.05 atm CO_2, 30 mM acetate, 50 mM lactate and calcium concentrations of 3–12 mM. Assuming that root mineral solubility is equivalent to pI_{HA} 54, the critical pH would be 5.66, 5.44, 5.31 and 5.22 at 3, 6, 9 and 12 mM Ca, respectively. Assuming a solubility equivalent to pI_{HA} 55, the corresponding values would be 5.51, 5.29, 5.16 and 5.08.

From experiment 1, it appeared that, on average, lesions progressed somewhat faster on surfaces from which the cementum had been ground than on intact root surfaces. This confirms the findings of McIntyre et al. [2000b], although the difference was much less than they reported, and deeper lesions were not found on all root surfaces. McIntyre et al. [2000b] suggested that removal

Downloaded from http://karger.com/cre/article-pdf/44/4/380/2499302/000318224.pdf by UniversitÃ¤tsbibliothek Bern user on 25 May 2023

of the cementum layer accelerated the initial rate of lesion progression, and that the rate was subsequently the same as on the intact surfaces. This suggestion is supported by the fact that, in the present study, at all pI_{HA}/pH, the groups showed similar mean differences between the ground and intact surfaces. If dentine and cementum showed significantly different solubilities, the difference between the ground and intact surfaces would be expected to increase as solution conditions became more undersaturated (i.e. as pI_{HA} increased). The mechanism proposed by McIntyre et al. [2000b] for cementum removal affecting only initial demineralization rate is not very convincing. A simpler explanation for a uniform difference is that the process of grinding off the cementum creates a smear layer and a subsurface region of mechanical damage which are more vulnerable to dissolution.

Magnesium, at a concentration high enough to produce supersaturation with respect to whitlockite, which is often associated with sclerosis, did not affect lesion depth. This is in striking contrast to the results of Paschalis et al. [1996], who found that dissolution of powdered dentine was strongly inhibited by magnesium at a much lower concentrations (0.5 mM). One possible explanation is that magnesium affected mineral loss but not lesion depth: ten Cate et al. [1998] observed such a phenomenon in the presence of fluoride. If this hypothesis is correct, then the continual removal by enzymic and mechanical processes in vivo – not modelled in this study – could considerably enhance the effect of inhibitors, by improving access to the deeper parts of the lesion. In any case, it is recommended that magnesium ions should be included in undersaturated buffers for studying root-surface lesion formation, since re-precipitation of magnesium-containing calcium phosphates could influence lesion formation [Daculsi et al., 1987; Shellis, 1994].

In conclusion, this work has shown that, under solution conditions similar to those in plaque fluid and with the overall pH range of 4.62 to 5.34, lesion formation on root surfaces is not influenced by the concentration of undissociated buffer acid, nor is it likely that the concentration of buffer acid anions is important. Lesion depth is not affected by the presence of magnesium at plaque fluid concentration. On surfaces from which the cementum had been removed, lesions were on average deeper than on intact surfaces, but this does not seem to reflect a difference in solubility. The results suggest that, while the solubility of the mineral of root tissues is raised, it is lower than has been suggested previously.

Downloaded from http://karger.com/cre/article-pdf/44/4/380/2499302/000318224.pdf by UniversitÃ¤tsbibliothek Bern user on 25 May 2023

References

Aamdal-Scheie A, Luan WM, Dahlén G, Fejerskov O: Plaque pH and microflora of dental plaque on sound and carious root surfaces. J Dent Res 1996;75:1901–1908.

Arends J, Christoffersen J, Ruben J, Christoffersen MR: Lesion progress in dentine and the role of fluoride; in Thylstrup A, Leach SA, Qvist V (eds): Dentine and Dentine Reactions in the Oral Cavity. Oxford, IRL Press, 1987, pp 117–126.

Ascenzi A: On the existence of bonds between ossein and inorganic bone fraction. Science 1950;112:84–86.

Bland JM, Altman DG: Measuring agreement in method comparison studies. Stat Methods Med Res 1999;8:135–160.

Budz JA, Lo Re M, Nancollas GH: The influence of high- and low-molecular weight inhibitors on dissolution kinetics of hydroxyapatite and human enamel in lactate buffers: a constant composition study. J Dent Res 1988;67:1493–1498.

Daculsi G, Legeros RZ, Jean A, Kérébel B: Possible physico-chemical processes in human dentin caries. J Dent Res 1987;66:1356–1359.

Damen JJ, Buijs MJ, ten Cate JM: Fluoride-dependent formation of mineralized layers in bovine dentin during demineralization in vitro. Caries Res 1998;32:435–440.

Edgar WM., Dodds MWJ, Higham SM: Changes in carboxylic acid and free amino acid profiles in human dental plaque after a carbohydrate challenge in situ. Biochem Soc Trans 1986;14:977.

Featherstone JDB, Rodgers BE: Effect of acetic, lactic and other organic acids on the formation of artificial carious lesions. Caries Res 1981;15:377–385.

Featherstone JDB, McIntyre JM, Fu J: Physicochemical aspects of root caries; in Thylstrup A, Leach SA, Qvist V (eds): Dentine and Dentine Reactions in the Oral Cavity. Oxford, IRL Press, 1987, pp 127–138.

Gray JA: Kinetics of enamel dissolution during formation of incipient caries-like lesions. Arch Oral Biol 1961;11:397–421.

Hals E, Nernaes A: Histopathology of in vitro caries developing around silver amalgam fillings. Caries Res 1971;5:58–77.

Higham SM, Edgar WM: Human dental plaque pH, and the organic acid and free amino acid profiles in plaque fluid, after sucrose rinsing. Arch Oral Biol 1989;34:329–334.

Hoppenbrouwers PMM, Driessens FCM: The effect of lactic and acetic acid on the formation of artificial caries lesions. J Dent Res 1988;67:1466–1467.

Hoppenbrouwers PMM, Driessens FCM, Borggreven JMPM: The vulnerability of unexposed human dental roots to demineralization. J Dent Res 1986;65:955–958.

Hoppenbrouwers PMM, Driessens FCM, Borggreven JMPM: The mineral solubility of tooth roots. Arch Oral Biol 1987;32:319–322.

Imfeld T: Evaluation of the cariogenicity of confectionery by intra-oral wire-telemetry. Helv Odont Acta 1977;21:1–29.

Lynch RJ, Mony U, ten Cate JM: The effect of fluoride at plaque fluid concentrations on enamel de- and remineralisation at low pH. Caries Res 2006;40:522–529.

Margolis HC, Moreno EC: Composition of pooled plaque fluid from caries-free and caries-positive individuals following sucrose exposure. J Dent Res 1992;71:1776–1784.

Margolis HC, Zhang YP, van Houte J, Moreno EC: Effect of sucrose concentration on the cariogenic potential of pooled plaque fluid from caries-free and caries-positive individuals. Caries Res 1993a;27:467–473.

Margolis HC, Zhang YP, Gewirtz A, van Houte J, Moreno HC: Cariogenic potential of pooled plaque fluid from exposed root surfaces in humans. Arch Oral Biol 1993b;38:131–138.

McIntyre JM, Featherstone JDB, Fu J: Studies of dental root surface caries. 1. Comparison of natural and artificial root surface caries. Austr Dent J 2000a;45:24–30.
McIntyre JM, Featherstone JDB, Fu J: Studies of dental root surface caries. 2. The role of cementum in root surface caries. Austr Dent J 2000b;45:97–102.
Paschalis EP, Tan J, Nancollas GH: Constant composition dissolution kinetics studies of human dentin. J Dent Res 1996;75:1019–1026.
Patel PR, Brown WE: Thermodynamic solubility product of human tooth enamel: powdered sample. J Dent Res 1975;54:728–736.
Ruben J, Arends J: Shrinkage prevention of in vitro demineralized human dentine in transversal microradiography. Caries Res 1993;27:262–265.
Schmidt WJ, Keil A: Polarizing Microscopy of Dental Tissues (translators: Poole DFG, Darling AI). Oxford, Pergamon, 1971.
Shellis RP: A microcomputer program to evaluate the saturation of complex solutions with respect to biominerals. Comput Appl Biosci 1988;4:373–379.
Shellis RP: Effect of a supersaturated pulpal fluid on the formation of caries-like lesions on the roots of human teeth. Caries Res 1994;28:14–20.
Shellis RP: A scanning electron-microscopic study of solubility variations in human enamel and dentine. Arch Oral Biol 1996;41:473–484.
Shellis RP, Heywood BR, Wahab FK: Formation of brushite, monetite and whitlockite during equilibration of human enamel with acid solutions at 37°C. Caries Res 1997;31:71–78.
ten Cate JM, Nyvad B, van de Plassche-Simons YM, Fejerskov O: A quantitative analysis of mineral loss and shrinkage of in vitro demineralized human root surfaces. J Dent Res 1991;70:1371–1374.
ten Cate JM, Buijs MJ, Damen JJ: pH-cycling of enamel and dentin lesions in the presence of low concentrations of fluoride. Eur J Oral Sci 1995;103:362–367.
ten Cate JM, Damen JJ, Buijs MJ: Inhibition of dentin demineralization by fluoride in vitro. Caries Res 1998;32:141–147.
Theuns HM, van Dijk JWE, Driessens FCM, Groeneveld A: The effect of undissociated acetic-acid concentration of buffer solutions on artificial caries-like lesion formation in human tooth enamel. Arch Oral Biol 1984;29:759–763.
Verbeeck RMH, de Bruyne PAM: The solubility behaviour of whitlockite at 25°C (abstract). J Dent Res 1989;68:1079.
Voegel JC, Gillmeth S, Frank RM: Calcium release from powdered enamel and synthetic apatite after pretreatment with various low molecular weight organic acids. Caries Res 1983;17:212–219.
Vogel GL, Zhang Z, Carey CM, Ly A, Chow LC, Proskin HM: Composition of plaque and saliva following a sucrose challenge and use of an α-tricalcium-phosphate-containing chewing gum. J Dent Res 1998;77:518–524.
Wefel JS, Clarkson BH, Heilman JR: Natural root caries: a histological and microradiographic evaluation. J Oral Pathol 1985;14:615–623.

Downloaded from http://karger.com/cre/article-pdf/44/4/380/2499302/000318224.pdf by UniversitÃ¤tsbibliothek Bern user on 25 May 2023