Copper in approximal plaque from conventional and non-gamma-2 amalgam restorations

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Differences in copper concentrations found in plaque adjacent to conventional compared with copper-rich non-gamma-2 amalgam fillings were evaluated. Plaque was sampled at baseline and up to 2 months after polishing of two to four fillings of each type of amalgam in six patients. Copper concentrations of several hundred ppm were found, with higher values in plaque sampled from copper-rich amalgam. However, the copper release from the copper-rich amalgam probably has very little influence on plaque ecology owing to a relatively low copper ion activity because of stable copper complexes in plaque. □ *Atomic absorption spectrometry; clinical study; metal release; operative dentistry*

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It has been shown that copper accumulated in dental plaque (1) reduces plaque acidogenicity (2–4) and inhibits the growth of mutans streptococci in vitro (5). Modern non-gamma-2 amalgams release more copper than conventional amalgams and especially at low pH (6). A lower prevalence of mutans streptococci in plaque from approximal non-gamma-2 amalgam fillings than in conventional amalgams has been reported (7), but the difference was not statistically significant. Since copper has a minimal bactericidal concentration for mutans streptococci around 400 ppm (5), it is possible that the accumulation of copper in plaque adjacent to the fillings did not reach bactericidal levels. The aim of this study was to measure the concentration of copper in plaque from approximal areas of conventional and non-gamma-2 fillings at different time intervals after polishing the fillings to conditions mimicking newly made restorations.

Materials and methods

Subjects

Six patients aged 35–55 years participated in the study. They had recently had two to four old class-II amalgam fillings in each quadrant replaced with non-gamma-2 amalgam (ANA 2000, Nordiska Affineriet, Helsingborg, Sweden) on one side of the dentition and conventional amalgam (STA 68, Ädelmetall AB, Malmö, Sweden) on the contralateral side. The copper content was 25% in ANA 2000 and 5.8% in STA 68. In all, 32 restorations with conventional amalgam and 34 restorations with non-gamma-2 amalgam were made. The mean age of the fillings at base line was 17.8 months (range, 10–27 months).

Plaque sampling

Samples were collected at base line and after periods of 1 and 2 months. At base line sampling the six patients were instructed not to brush their teeth during the preceding 24 h. Plaque samples were collected from the buccal and lingual sides of the interproximal areas with restoration. Sterile plastic carvers were used, to avoid metallic contamination from the fillings. The samples were pooled in small glass tubes, one for each side in the dentition. Before use the tubes had been washed in 65% nitric acid (HNO_3), rinsed in distilled water, vacuum-dried for 7 days, and

Table 1. Concentration of copper in biopsy specimens of approximal gingiva adjacent to sound surfaces or to surfaces filled with conventional (STA 68) or non-gamma-2 (ANA 2000) amalgam in six subjects ($\bar{x} \pm$ SD)

Surface adjacent to specimens	Total Cu concentration (μg)	(μm/mg)
Sound	0.012 ± 0.003	0.032 ± 0.061
Filled		
STA 68	0.157 ± 0.216	0.205 ± 0.257
ANA 2000	0.082 ± 0.060	0.115 ± 0.096

weighed five times during this period. The plaque on the carver was transferred to the glass tube with a small sterile plastic pin. The glass tubes with plaque were vacuum-dried for 7 days and weighed five times, the first time after 48 h. At the base-line sampling small biopsy specimens of gingival tissue were excised from areas in close contact with conventional and non-gamma-2 amalgam fillings. One specimen was also excised in each subject from gingival tissues with no adjacent filling. The specimens were placed in the same kind of glass tubes used for plaque and then vaccum-dried to constant weight as described previously. All approximal areas of the test fillings were then thoroughly polished, using pumice and Sof-Lex (3M Co) finishing strips to mimic the condition of newly made fillings.

The patients were told to refrain from interproximal cleaning of the test teeth during 1 month. The test fillings were polished as described above. Afterwards the patients were told to refrain from interproximal cleaning of the test teeth for 2 more months. Plaque was again collected, and finally all teeth were thoroughly polished.

Determination of copper

Plaque and tissue samples were dried at 60°C until constant weight, and then the dry weight was determined. An aliquot of 100 μl concentrated HNO_3 was added to each sample, which was then kept at 60°C until all HNO_3 had evaporated. A volume of 200 μl HNO_3 was added, and the samples diluted with 1.8 ml deionized H_2O. The amount of copper was determined by flameless atomic absorption spectrometry in accordance with the manufacturer's manual (Perkin-Elmer, USA).

Statistical analysis

Differences between the concentration of copper in biopsy specimens and the total plaque amount of copper were analyzed using Student's *t* test.

Results

Because of the very small sample sizes, copper values are given as both total copper and copper concentrations. The amount of copper in approximal gingiva was higher in specimens taken adjacent to surfaces restored with conventional amalgam than in non-gamma-2 amalgam (Table 1). The difference was not statistically significant.

The amounts of copper found in approx-

Table 2. Copper in plaque from surfaces filled with conventional (STA 68) or non-gamma-2 (ANA 2000) amalgam ($\bar{x} \pm$ SD)

	Total Cu (μg)		Total Cu per total plaque amount of all samples (μg Cu/mg plaque)	
	STA 68	ANA 2000	STA 68	ANA 2000
Base line	0.186 ± 0.250	0.264 ± 0.323	0.317	0.716
1 month	0.069 ± 0.047	0.202 ± 0.245	0.160	0.957
2 months	0.011 ± 0.004	0.044 ± 0.084	ND*	ND

* Not determined.

imal plaque are shown in Table 2. Plaque samples collected at base line from the fillings made about 1½ years earlier showed higher copper contents than samples taken 1 or 2 months after polishing of the restorations. The copper content in plaque was higher in plaque sampled from non-gamma-2 restorations than from conventional amalgam fillings at the three examinations, but the differences were not statistically significant. At the 2-month examination very small sample volumes could be collected, and two samples gave negative values on dry weight.

Discussion

Copper analysis using flameless atomic absorption has a good sensitivity, with a detection limit in our hands of approximately 0.01 μg/ml sample.

There was a marked difference in copper release to gingiva and to plaque. A higher copper release to gingiva from conventional amalgam may be due to a high initial degradation of this material in the oral cavity. The opposite tendency for copper in plaque may be explained by a higher long-term release from high copper-containing non-gamma-2 amalgam. It seems likely that copper concentrations in plaque reflect actual release better than gingiva concentrations, owing to the more dynamic interaction with oral fluids. High copper concentrations in plaque found immediately after placement of fillings may therefore have been washed away, whereas this has not occurred in gingival tissue. However, the differences were in all instances fairly small.

Copper concentration in plaque exceeded 1000 ppm at base line and was still several hundred ppm 1 month later, with higher values in plaque from non-gamma-2 amalgam restorations than in plaque from conventional amalgam fillings. This observation is in line with in vitro findings (6) demonstrating that more copper was released from non-gamma-2 amalgams than from conventional ones over a 30-day period and that values of around 300 ppm copper were recorded in solutions containing high copper amalgam at pH 4–5. The minimal bactericidal concentration of copper is around 400 ppm for mutans streptococci (5).

It should be noted that the copper concentrations found reflect total copper. Conceivably, a major part of this will be firmly bound in plaque, and therefore only an minor fraction is probably available as free copper ions, thus limiting the influence on plaque ecology. The small sample sizes in this study precluded an evaluation of this point. Copper ions released over a prolonged period may form stable ionic interactions with polyanions (such as LTA) or covalent bindings with atoms having unshared electron pairs like N, O, or S (8, 9).

Studies on copper in mouth rinses have indicated plaque copper concentrations of less than 250 ppm to be of little influence on plaque metabolism, whereas at higher levels acid formation is affected (4). However, a direct inhibitory effect on growth of mutans streptococci in continuous culture experiments has been noted when copper at extremely low concentrations (0.16 mM, approximately 10 ppm) has been added (10). Furthermore, dental plaque is considered a rather anaerobic environment (11), and in such a millieu copper has been shown to be more effective against mutans streptococci than under aerobic conditions (12). The minor effect on the proportion of mutans streptococci in plaque from non-gamma-2 restorations (7) may therefore not exclude the possibility that copper released from such amalgam fillings can exert a cariostatic effect and contribute to a reduction of the risk of secondary caries.

References

1. Afseth J, Oppermann RV, Rölla G. Accumulation of Cu and Zn in human dental plaque *in vivo*. Caries Res 1983;17:310–4.
2. Afseth J, Oppermann RV, Rölla G. The *in vivo* effect of glucose solutions containing Cu^{++} and Zn^{++} on the acidogenicity of dental plaque. Acta Odontol Scand 1980;38:229–33.
3. Oppermann RV, Johansen JR. The effect of fluoride salts of copper, silver and tin on the acidogenicity of dental plaque *in vivo*. Scand J Dent Res 1980;88:476–80.
4. Afseth J. Some aspect of the dynamics of Cu and Zn retained in plaque as related to their effect on plaque pH. Scand J Dent Res 1983;91:169–74.

5. Maltz M, Emilson CG. Susceptibility of oral bacteria to various fluoride salts. J Dent Res 1982;61: 786–90.
6. Derand T, Johansson B. Corrosion of non-γ_2-amalgams. Scand J Dent Res 1983;91:55–60.
7. Wallman-Björklund C, Svanberg M, Emilson CG. *Streptococcus mutans* in plaque from conventional and from non-gamma-2 amalgam restorations. Scand J Dent Res 1987;95:266–9.
8. McGilvery RV. Biochemistry—a functional approach. Philadelphia: WB Saunders Co, 1970: 147–8.
9. Afseth J. Biological effects of Cu^{++} and Zn^{++} in the mouth [thesis]. Oslo: University of Oslo, 1983.
10. Aranha H, Strachan RC, Arceneaux JEL, Byers BR. Effect of trace metals on growth of *Streptococcus mutans* in a teflon chemostat. Infect Immun 1982;35:456–60.
11. Tenovuo J, Valtakoski J. The correlation between salivary peroxidase activity, salivary flow rate, and the oxidation-reduction potentials of human saliva and dental plaque suspensions. Acta Odontol Scand 1976;34:169–76.
12. Evans SL, Tolbert C, Arceneaux JEL, Byers BR. Enhanced toxicity of copper for *Streptococcus mutans* under anaerobic conditions. Antimicrob Agents Chemother 1986;29:342–3.

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