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Nickel in dental plaque and saliva in patients with and without orthodontic appliances

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SUMMARY The aim of this study was to compare the content of nickel in the saliva and dental biofilm in young patients with and without orthodontic appliances. The possible influence of a dietary intake of nickel on recorded nickel levels was examined.

Nickel content in unstimulated whole saliva and in dental plaque of 24 boys and girls (mean age 14.8 years) with intraoral fixed orthodontic appliances was compared with 24 adolescents without such an appliance. Sample collection was set up to exclude nickel contamination. Diet intake was recorded for the preceding 48 hours to account for the influence of recent nickel content in food. Saliva and plaque were analysed for nickel content using an electrothermal atomic absorption spectrometric (ETAAS) method. The acidified saliva samples were analysed as Millipore-filtered saliva with filter-retained fractions and plaque following dissolution in acids.

No significant difference in nickel content of filtered saliva was found between the test and the control samples ($P = 0.607$); the median values of nickel content were 0.005 and 0.004 μg/g saliva, respectively. On the other hand, a significant difference was found for the filter-retained fraction ($P = 0.008$); median values for nickel were 25.3 and 14.9 μg/g, respectively. A significant difference in nickel content between test and control samples was also found in plaque collected at various tooth sites ($P = 0.001$; median values 1.03 and 0.45 μg/g, respectively). A stronger difference was found when comparing plaque collected from metal-covered tooth surfaces than from enamel surfaces of orthodontic patients. No association could be found between calculated dietary intake of nickel and recorded nickel in the test and control samples.

It is concluded that nickel release occurs into the dental plaque and components of saliva of orthodontic patients, a situation that may reflect time dependence of its release from orthodontic appliances into the oral cavity and an aggregation of nickel at plaque sites.

Introduction

Nickel is a strong sensitizer and one of the most common causes of contact allergies (Nielsen and Menné, 1993). Patients and parents therefore may express concern about possible leakage of metal ions from an orthodontic appliance.

In vitro release of nickel from orthodontic appliances has been noted using microscopic analysis of corrosion as well as chemical analyses of orthodontic components when exposed in an artificial oral environment (Park and Shearer, 1983; Kratzenstein *et al*., 1988; Grimsdottir *et al*., 1992; Kerosuo *et al*., 1995; Jia *et al*., 1999). When incubated in artificial saliva, orthodontic appliances of various types release 22–40 μg nickel per day, compared with the estimated dietary intake of between 100 and 800 μg per day [International Programme on Chemical Safety (IPCS), 1991]. Release of nickel is reported to vary with composition and manufacturing of the appliance components (Grimsdottir *et al*., 1992) and between archwire alloys and mechanical straining (Jia *et al*., 1999) but not actual nickel content (Grimsdottir *et al*., 1992).

Data from studies *in vivo* on nickel content in saliva as a result of insertion of orthodontic components are inconclusive (Kocadereli *et al*., 2000; Eliades *et al.,* 2003). Overall no difference has been demonstrated between individuals with or without orthodontic appliances, or between saliva collected before and after appliance insertion (Gjerdet *et al*., 1991; Kerosuo *et al.*, 1997; Agaoglu *et al.*, 2001). However, both Gjerdet *et al.* (1991) and Agaoglu *et al*. (2001) demonstrated a significant, albeit inconsistent, difference during periods of orthodontic treatment. Furthermore, Kratzenstein *et al*. (1988) found nickel content in saliva from patients to vary between <0.001 and 0.19 μg/ml during different phases of orthodontic treatment. Those investigators, however, concluded that dietary influences and variations in saliva flow and dilution may have biased their results. Bishara *et al.* (1993) reported elevated blood levels after insertion of orthodontic appliances, but concluded that contamination at sampling and diet were likely to explain the variations. Thus, the influence of orthodontic appliances on nickel content in saliva remains unclear.

The warm and moist aerobic condition in the mouth offers an aggressive environment for electrolytic and electrochemical activity. Because orthodontic appliances will render regular oral hygiene procedures more difficult, a dental biofilm accumulates on appliance components and adjacent tooth surfaces in most patients (e.g. Lundström

et al., 1980; Hamp *et al*., 1982). The older and thicker the biofilm growth, the greater the increase in the anaerobic conditions. This environment encourages the corrosion of metals (Hamilton, 1998). The anaerobic condition in older biofilms may favour corrosion of the underlying nickel alloy, and changing microbial ecology and biological debris may also contribute. Furthermore, release of ionic nickel may not be linear, but may have an additive effect (Eliades *et al*., 2003). Nickel released from an appliance may complex with older ions or glycoproteins in the dental biofilm and thus accumulate.

Besides originating from orthodontic appliances, nickel may be accumulated from saliva and food. In individuals without orthodontic appliances, the nickel content in whole stimulated saliva has been reported to be 8.2 μg/l (Gjerdet *et al*., 1991), and in parotid saliva 1.9 ± 1.0 μg/l (Catalanatto and Sunderman, 1977). Presumably, the nickel content in glandular saliva, like nickel in other body fluids, is largely influenced by dietary intake. Absorbed dietary nickel will have an elimination half time of 28–29 hours (Sunderman *et al*., 1989).

The aim of this study was to compare the content of nickel in the saliva and the dental biofilm in young patients with and without orthodontic appliances. The possible influence of dietary intake of nickel on recorded nickel levels was examined.

Study cohort

Healthy children and adolescents with an intra-oral fixed orthodontic appliance in one or both arches who were scheduled for a routine check-up at the Department of Orthodontics, Dental School of Umeå, Sweden, were eligible for the test group. A control group without any type of metal appliance or restoration in the mouth was selected from patients referred to a dental hygienist at the Public Dental Health Service in Umeå for oral hygiene instruction. The exclusion criteria in both groups were (1) disease/medication and (2) intraoral piercing/metal restorations.

As the presence of biofilm was a necessity for sampling, individuals eligible for the test and control groups were screened visually before any treatment procedures were undertaken. Those who had visible biofilm were given written information and asked if they would volunteer to undergo biofilm and saliva sampling at the visit. For participation, written consent by the patient or parent was required. If requested within two days, the patients or their parents were allowed to have the collected samples destroyed. The samples were taken before and after noon, but dietary intake was not accepted within 1 hour before sampling.

In total, 14 boys and 10 girls, from 11.0 to 19.1 years (mean age 14.8 years), agreed to participate in the study. The average period since appliance insertion was 16 months at the time of sample collection. All patients were bonded with stainless steel brackets in one or both arches, and six (25 per cent) also had stainless steel orthodontic bands (Unitek/3M, Monrovia, California, USA) on their upper and lower first molars. Eight patients (33 per cent) had a nickel–titanium alloy archwire (Nitinol© or NiTi© arch-wires) at the time of sampling, and the remaining 16 participants had stainless steel wires (archwires from Unitek/3M, and NiTi© from Ormco Corporation, Orange, California, USA).

Eleven boys and 13 girls aged 8.3–18.9 years (mean age 14.8 years) agreed to participate in the control group.

Methods

A blank test of the collecting sticks and test tubes was undertaken by leaving them in a few millilitres of Millipore water for 1 hour and 24 hours, respectively, and by analysing the acidified water for nickel content as described below. No nickel was found to have been released from these objects.

Saliva collection

The participants were asked to rinse their mouth for 30 seconds with 10 ml of distilled water and to rest for two minutes before saliva was collected. Approximately 2 ml of unstimulated whole saliva was collected into pre-weighed plastic polyprophylene tubes (ref. 62.554.502, lot 1127 1501, Sarstedt, Nümbrecht, Germany). The tubes and funnels had been tested to ensure that they were free of nickel, and talcum-free gloves were used when handling the tubes. The samples were stored at −20°C for later analysis.

Plaque collection

Dental biofilm was sampled with nickel-free plastic sticks (Quick-Stick, Dentonova AB, Stockholm, Sweden). The samples were transferred to pre-weighed nickel-free 1.5 ml polyprophylene tubes (ref. 72.690, control no. 0681 0222016, Sarstedt). The wet weight of the sample was calculated, and the samples were stored at −20°C until analysis.

For the orthodontic patients, separate biofilm samples were taken, if feasible, from two sites: (1) metal surfaces of orthodontic molar bands or brackets, and (2) enamel surfaces without direct contact with the appliance. Sampling was not carried out where there was gingival bleeding. The mean amount of plaque sampled from the test and the control patients was approximately 2 μg (range 0.1–5.4).

Dietary history

In order to reveal recent extremes of nickel exposure from diet or smoking, the participants were asked to answer a questionnaire on food intake and smoking during the previous 48 hours. The patients reported on the type, frequency, and approximate amount (classified by the participants as ‘small’, ‘moderate’ or ‘large’) of food and drink at and between meals. Based on reported intakes weighted for nickel content in various food/beverage items (IPCS, 1991; Livsmedelsverket, 1997), nickel intake was

estimated during specific time windows (0–3, 4–6, 7–12, 13–24 and 25–48 hours) preceding saliva and biofilm sampling. This allowed for ranking the participants into those with a low, moderate or high nickel intake as well as statistical testing for a possible relationship between nickel in saliva and plaque samples and food intake.

Nickel determinations

The biofilm samples were diluted in water and acids (H_2O:HNO_3:HCl, 5:4:1), kept at 60°C for 4 hours to dissolve nickel before analysis, and stored overnight. The saliva samples were diluted with Millipore water and filtered through a 5 μm white SMWP Millipore filter (Millipore Corporation, Bedford, Massachusetts, USA). Approximately 0.7 g of the saliva was acidified in nitric acid. The filter and its retained fractions were dried at room temperature overnight, weighted, and dissolved by heating in nitric and hydrochloric acids (HNO_3:HCl, 4:1). If necessary the samples were diluted in 1 per cent nitric acid before analysis.

Nickel content was determined using electrothermal atomic absorption spectrometry (ETAAS) with a transverse heated electrothermal atomizer (THGA) with longitudinal Zeeman effect background correction (Perkin-Elmer Model 4100 ZL, Perker-Elmer GmhB, Überlingen, Germany). All manipulations during sample pre-treatment were performed in a laminar flow clean bench (class 100 working environment). A more detailed description of these procedures has been published (Slanina *et al.*, 1985). All nickel determinations were performed at the Department of Analytical Chemistry, Umeå University. Calibration curves from acidified water standards were normally used as standard addition showed 100 per cent recovery. The detection limit of the method for unfiltered samples, based on ×3 the standard deviation for nickel concentrations in blank solutions, was 0.001–0.002 μg/g. For biofilm samples the detection limit was typically ×5 higher due to increased blank concentrations.

Pilot study

Data on nickel in dental biofilm for a pilot test and a control group where the methods given above were used is also reported. Samples of biofilm were taken from 14 patients under orthodontic treatment who apparently neglected their oral hygiene. These participants were all under treatment with a full-bonded appliance and had a mean age of 16.2 years (range 10.3–23.4) at sample collection. Control samples were taken from 14 patients without an appliance visiting a dental hygienist as described above (mean age of 13.8 years, range 7.3–19.2). Procedures for collecting biofilm were identical to those described above.

Statistical analysis

The distributions of nickel content in the saliva and biofilm were skewed. Thus median and range values are given and non-parametric tests were applied. The Mann–Whitney two-sample test was used to assess differences in saliva and biofilm nickel content between the test and control groups. The Wilcoxon signed rank test was used to determine differences in biofilm nickel content within surfaces of orthodontically treated individuals. A multiple regression model was used to test for associations between the dependent (i.e. saliva, plaque) and independent (age, gender, diet) variables. Statistical analysis was undertaken using SPSS v. 11.5 statistical package (Statistical Package for the Social Sciences, SPSS Inc., Chicago, Illinois, USA). $P < 0.05$ was considered significant.

Ethic approval

The study was approved by the committee on research ethics at Umeå University (§109/02, 02 091).

Results

The nickel content in filtered saliva was very low (< 0.08 μg/g) in both the test and control groups (Table 1). However, the content of nickel in the saliva 'debris' retained on the filters, which was 1000-fold higher than in the saliva filtrate, was significantly higher ($P < 0.01$) in orthodontic patients compared with the controls.

A large variation in saliva nickel content in both groups (Figure 1), but no association with estimated nickel exposure from diet for the group or for outliers in the filtered saliva sample.

Individuals with orthodontic appliances also had a significantly higher nickel content in the dental plaque samples compared with the controls regardless of whether samples were harvested from tooth surfaces in close proximity to ($P < 0.001$) or further away from the orthodontic appliance ($P < 0.05$; Table 2). Nickel content in samples from orthodontic patients was ×2 higher in biofilms covering metal surfaces than distant enamel surfaces (Table 2). In line with this, plaque from metal surfaces showed a higher median nickel content than that collected from enamel surfaces at a distance from a metal-covered tooth surface ($n = 18$; $P = 0.014$). When limiting such an analysis to an intra-individual comparison of metal-covered and enamel surfaces in orthodontic patients, a significant difference was still found ($n = 12$; $P = 0.007$).

Table 1 Nickel content in Millipore-filtered saliva (median and range, μg/g) and in saliva sediment, in controls and test patients.

Samples (μg/g)		Controls ($n = 24$)	Patients ($n = 24$)	P-value
Filtered saliva	Median	0.004	0.005	0.607
	Range	0.001–0.084	0.001–0.037	
Saliva sediment	Median	14.85	25.25	0.008
	Range	2.00–39.30	9.61–51.20	

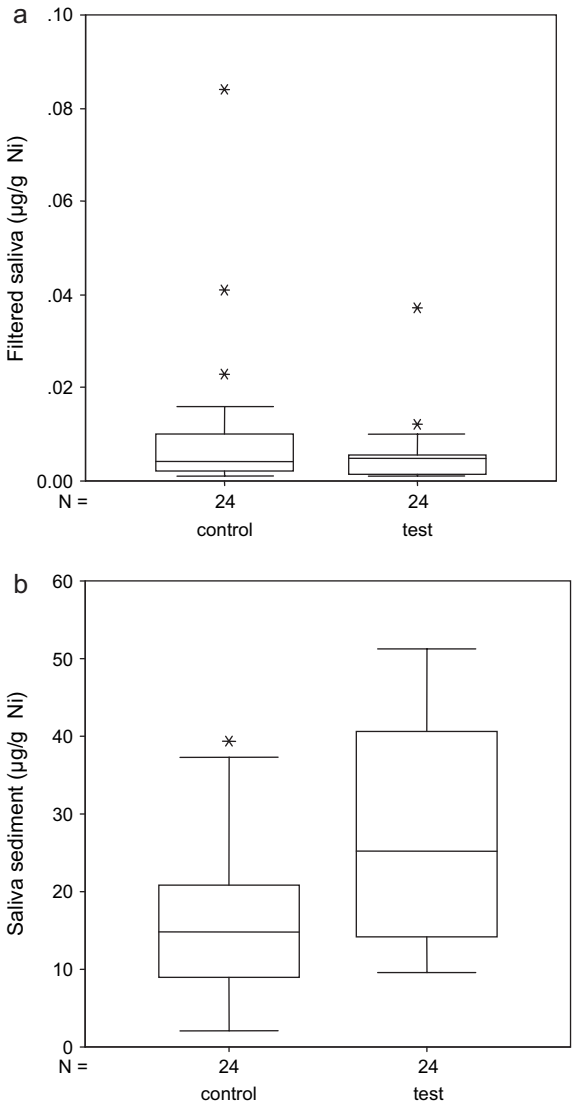

Figure 1 Boxplot graphs of nickel content in the control and test subjects (a) filtered saliva and (b) saliva sediment to illustrate variation in nickel content (µg/g). Outliers marked with an asterisk.

Comparison of nickel in the test and control groups from the pilot study also showed a significantly higher nickel content in the biofilm of orthodontic patients ($P = 0.007$; Table 3). Notably, the nickel levels in the pilot study, where the biofilm was more mature, were on average more than ×2 higher per weight unit in both patients and controls compared with the main study.

Nickel content in saliva filtrate, saliva debris retained on the filter, or in biofilm samples was unrelated to estimated exposure from diet during the previous 48 hours. None of the participants admitted in the questionnaire to being a smoker.

Discussion

Nickel content in filtered saliva was found to be low and close to detection level, and without a difference between patients and their controls. In contrast, a higher nickel content in the dental plaque as well as in the filter-retained fraction of saliva was found in patients with orthodontic appliances compared with their non-appliance controls. These observations *in vivo* are thus in line with several reports from *in vitro* corrosion studies (e.g. Park and Shearer, 1983; Jia *et al.*, 1999) in which a release of nickel from orthodontic appliances has been demonstrated. Recently, the presence of nickel and cobalt has been demonstrated in oral mucosa cells of orthodontics patients (Faccioni *et al*., 2003).

A significantly higher nickel content was found in the intra-individual comparison of plaque taken from metal surfaces (band and brackets) than from enamel surfaces in subjects with an appliance. The oral plaque composition of micro-organisms and biological debris may be a further local environmental factor favouring corrosion of the underlying nickel alloy in the corrosion-aggressive oral environment. Because a greater ion release from orthodontic brackets has been found at a lower pH (Huang *et al*., 2001), a direct nickel release by the acidogenic plaque on metal surfaces is likely when plaque responds to pH changes in the oral cavity; the amount of this release could be greater because it is unclear whether salivary buffering substances will significantly influence pH changes in the depth of the plaque (Tenovuo and Lagerlöf, 1994).

In contrast to earlier negative reports of nickel in saliva of orthodontic patients, the results of the present study demonstrated nickel in the filter-retained fraction of whole unstimulated saliva. In addition to being incorporated into dental plaque, nickel released from dental alloys is likely to be, at least temporarily, attached to epithelial cells of the mucosa, to bacteria, and to large salivary macromolecules, all of which are normally present in whole saliva. Unlike earlier studies, in the present investigation the saliva was filtered because it was found that digestion at an elevated temperature in the presence of acids was insufficient to provide a homogenous solution with respect to nickel. Because of the filter size (5 µm), the filter-retained fraction of the sampled saliva is supposed to contain nickel in mucosal debris of sloughed cells with attached large proteins and bacteria or possibly aggregates of micro-organisms. This aggregation of nickel in 'salivary sediment' may explain the finding of a high nickel content in the filter-retained components of saliva, as well as earlier attempts (e.g. Bishara *et al*., 1993) to explain large inter-individual variations in salivary nickel.

Collection of saliva in previous studies of nickel has mainly been carried out two or more hours following food intake to standardize flow rate and to avoid an influence on nickel content by diet. If occurring, nickel release by corrosion into the saliva is more likely to take place shortly after a meal due to the sharp fall in pH within minutes, which is in general not normalized until 30–60 minutes after intake (Nyvad and Fejerskov, 1994). This further supports the supposition and findings that released nickel will not be found to any major extent in filtered saliva but in the plaque and salivary

Table 2 Nickel content in the biofilm (dental plaque; median and range, μg/g) from enamel surfaces in controls and for test patients with an orthodontic appliance separated for collection sites.*

Samples		Controls	Patients	*P*-value
Enamel surfaces in controls and all collection sites in patients	*n**	23	24	<0.001
	Median	0.445	1.025	
	Range	0.110–2.130	0.250–4.347	
Enamel surfaces in controls and metal surfaces in patients	*n**	23	18	0.001
	Median	0.380	1.430	
	Range	0.110–2.130	0.250–5.770	
Enamel surfaces in controls and enamel surfaces in patients	*n**	23	18	0.047
	Median	0.380	0.685	
	Range	0.110–2.130	0.220–2.370	

*Number of subjects varies depending on availability of plaque at collection sites.
P-value for a difference between metal and enamel surfaces in patients: $P = 0.014$.

Table 3 Nickel content in dental plaque (median and range, μg/g) in test patients and controls in the pilot study.

Samples		Controls ($n = 14$)	Patients ($n = 14$)	*P*-value
Enamel surfaces of controls and metal surfaces in patients	Median	0.875	2.690	0.007
	Range	0.41–2.07	0.33–6.90	

sediment. As has been found for fluorides following oral rinsing (Tatevossian, 1990; Larsen and Bruun, 1994), the results of the present study show that it is likely that nickel released into the saliva will aggregate in plaque.

The demonstration of nickel in the filter-retained fraction of whole saliva and in the dental plaque may thus reflect the dynamics of nickel release from the appliances into the oral cavity with an aggregation over time of nickel from the saliva in the plaque. As a comparison, fluoride levels in plaque are stated to be usually ×50–200 higher than in whole saliva (Larsen and Bruun, 1994). Because older plaque characterized the test pilot sample, the test data with its higher nickel content is reported (Table 3) to support the supposition of nickel aggregation in the plaque.

An individual variation in newly secreted salivary nickel is to be expected because serum nickel is influenced by food, tobacco smoking, and nickel in air and water (IPCS, 1991). The release of nickel ions into the saliva by corrosion is also likely to vary over time, depending on factors shown to influence ion release, such as mechanical stress in the appliance (Jia *et al*., 1999) and pH levels (Huang *et al*., 2001). Large variations in nickel content of saliva have been found in earlier studies of orthodontic patients. Kerosuo *et al*. (1997) noted differences from 0 to 240 μg/ml and a variation, although smaller, was also found in the present samples. As pointed out by Kerosuo *et al*. (1997) as well as Eliades *et al*. (2003), the sampling methods used in studies of salivary nickel in orthodontic patients are so far limited to describing the momentary total concentration of nickel in the saliva following wetting of teeth and oral mucous membranes after secretion, as in the present study. The elimination half-time into urine for both nickel absorbed from drinking water and food has been given as 28 ± 9 hours (Niebor *et al*., 1992). Even if a more detailed 48-hour report on diet had been used, only a rough assessment of nickel exposure from diet could have been made due to the limited information available on nickel content in various food (IPCS, 1991; Livsmedelsverket, 1997). Because no influence of calculated dietary nickel intake could be demonstrated on nickel content of saliva and plaque for any of the examined time periods, it was concluded that the outcomes of differences in salivary nickel between the groups were not explained by differences in dietary intake.

Nickel in saliva and plaque may be released, besides by corrosion of the appliance, by abrasion during fabrication or later by chewing. The polished surfaces of new orthodontic appliance components will show metal particles, which are likely to be found in saliva of recently banded/bonded patients. The insoluble precipitate, causing large variations of the results and therefore not included in the final analyses by Kerosuo *et al*. (1997) and Agaoglu *et al*. (2001), may have to some extent included metal particles from polished surfaces of new appliances, a condition that suggests a need for analyses of essentially 'aged' appliances as in the present investigation. Whether nickel found in the current research originates from the appliances by abrasion during normal mastication has to be clarified in future studies.

Although nickel is a strong sensitizer in contact allergy development (Nielsen and Menné, 1993) adverse reactions related to orthodontic appliances are rare (e.g. Schuster *et al*., 2004). This could be explained by a possible tolerance development by early intraoral exposure to nickel, which

has been hypothesized and given some support by experimental data (Vreeburg *et al*., 1984; Kerosuo *et al*., 1997). The present demonstration of nickel release from orthodontic appliances, in contrast to earlier mentioned inconclusive *in vivo* investigations, can be seen in such a context, stressing the need for further studies of oral exposure to nickel and the nature of nickel in plaque.

Conclusion

A significantly higher content of nickel was found in the plaque and the filter-retained fraction of whole saliva of patients with orthodontic appliances compared with non-orthodontic patients. Moreover, in orthodontic patients, a significantly higher nickel content was found in plaque from metal surfaces (band and brackets) than from enamel surfaces. This value appears to increase with the age of the biofilm. An influence on the outcome of dietary intake of nickel by meals was rejected. The findings are considered to reflect a time dependence of nickel release from orthodontic appliances into the oral cavity with an accumulation of nickel in the dental biofilm.

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References

Agaoglu G, Arun T, Izgi B, Yarat A 2001 Nickel and chromium levels in the saliva and serum of patients with fixed orthodontic appliances. Angle Orthodontist 71: 375–379

Bishara S E, Barret R D, Selim M I 1993 Biodegradation of orthodontic appliances. Part II. Changes in the blood level of nickel. American Journal of Orthodontics and Dentofacial Orthopedics 103: 115–119

Catalanatto F, Sunderman Jr F W 1977 Nickel concentrations in human parotid saliva. Annals of Clinical and Laboratory Science 7: 146–151

Eliades T, Trapalis C, Eliades G, Katsavrias E 2003 Salivary metal levels of patients: a novel methodological and analytical approach. European Journal of Orthodontics 25: 103–106

Faccioni F, Franceschetti P, Cerpelloni M, Fracasso M E 2003 *In vivo* study on metal release from fixed orthodontic appliances and DNA damage in oral mucosal cells. American Journal of Orthodontics and Dentofacial Orthopedics 124: 687–693

Gjerdet N R, Erichsen E S, Remlo H E, Evjen G 1991 Nickel and iron in saliva of patients with fixed orthodontic appliances. Acta Odontologica Scandinavica 49: 73–78

Grimsdottir M R, Gjerdet N R, Hensten-Pettersen A 1992 Composition and *in vitro* corrosion of orthodontic appliances. American Journal of Orthodontics and Dentofacial Orthopedics 101: 525–532

Hamilton W A 1998 Bioenergetics of sulphate-reducing bacteria in relation to their environmental impact. Biodegradation 9: 201–212

Hamp S E, Lundström F, Nyman S 1982 Periodontal condition in adolescents subjected to multiband orthodontic treatment with controlled oral hygiene. European Journal of Orthodontics 4: 77–86

Huang T H, Yen C C, Kao C T 2001 Comparison of ion release from new and recycled orthodontic brackets. American Journal of Orthodontics and Dentofacial Orthopedics 120: 68–75

International Programme on Chemical Safety (IPCS) 1991 Environmental Health Criteria 108. Nickel. World Health Organization, Geneva

Jia W *et al.* 1999 Nickel release from orthodontic archwires and cellular immune response to various nickel concentrations. Journal of Biomedical Materials Research (Applied Biomaterials) 48: 488–495

Kerosuo H, Moe G, Kleven E 1995 *In vitro* release of nickel and chromium from different types of simulated orthodontic appliances. Angle Orthodontist 65: 111–116

Kerosuo H, Moe G, Hensten-Pettersen A 1997 Salivary nickel and chromium in subjects with different types of fixed orthodontic appliances. American Journal of Orthodontics and Dentofacial Orthopedics 111: 595–598

Kocadereli L, Atac P A, Kale P S, Özer D 2000 Salivary nickel and chromium in patients with fixed orthodontic appliances. Angle Orthodontist 70: 431–434

Kratzenstein B, Koppenburg P, Sauer K H, Weber H 1988 Speichelanalysen kieferorthopädischer Patienten zum nachweis geloster metallischer Bestandteile. Quintessenz 39: 693–703

Larsen M, Bruun C 1994 Caries chemistry and fluoride – mechanism of action. In: Thylstrup A, Fejerskov O (eds) Textbook of clinical cariology, 2nd edn. Munksgaard, Copenhagen

Livsmedelsverket 1997 Allergiinformation nr 7. Nickel, krom och andra mineralämnen. Livsmedelsverket, Stockholm

Lundström F, Hamp S E, Nyman S 1980 Systematic plaque control in children undergoing long-term orthodontic treatment. European Journal of Orthodontics 2: 27–39

Nieboer E, Sanford W E, Stace B C 1992 Absorption, distribution and excretion of nickel. In: Nieboer E, Nriagu J O (eds) Nickel and human health; current perspective. Wiley & Sons, New York

Nielsen N H, Menné T 1993 Nickel sensitization and ear piercing in an unselected Danish population. Glostrup allergy study. Contact Dermatitis 29: 16–21

Nyvad B, Fejerskov O 1994 Development, structure and pH of dental plaque. In: Thylstrup A, Fejerskov O (eds) Textbook of clinical cariology, 2nd edn. Munksgaard, Copenhagen

Park H Y, Shearer T R 1983 *In vitro* release of nickel and chromium from simulated orthodontic appliances. American Journal of Orthodontics 84: 156–159

Schuster G, Reichle R, Bauer R R, Schopf P M 2004 Allergies induced by orthodontic alloys: incidence and impact on treatment. Results of a survey in private orthodontic offices in the Federal State of Hesse, Germany. Journal of Orofacial Orthopedics 65: 48–59

Slanina P, Frech W, Bernhardson Å, Cedergren A, Mattsson P 1985 Influence of dietary factors on aluminium absorption and retention in the brain and bone of rats. Acta Pharmacologica et Toxicologica 56: 331–336

Sunderman Jr F W, Hopfer S M, Sween K R, Marcus A H, Most B M, Creason J 1989 Nickel absorption and kinetics in human volunteers. Proceedings of the Society for Experimental Biology and Medicine 191: 5–11

Tatevossian A 1990 Fluoride in dental plaque and its effects. Journal of Dental Research 69 (Special issue): 645–652

Tenovuo J, Lagerlöf F 1994 Saliva. In: Thylstrup A, Fejerskov O (eds) Textbook of clinical cariology, 2nd edn. Munksgaard, Copenhagen

Vreeburg K J J, De Groot K, von Blomberg B M E, Scheper R J 1984 Induction of immunological tolerance by oral administration of nickel and chromium. Journal of Dental Research 63:124–128