

Nickel ion concentrations in the saliva of patients treated with self-ligating fixed appliances: a prospective cohort study

Nickelionenkonzentration im Speichel von Patienten mit selbstligierenden festsitzenden Apparaturen: Eine prospektive Kohortenstudie

Lina Gölz¹ · Anna Christine Knickenberg² · Ludger Keilig^{2,3} · Susanne Reimann² · Spyridon N. Papageorgiou^{1,2} · Andreas Jäger¹ · Christoph Bourauel²

Received: 16 March 2015 / Accepted: 28 July 2015 / Published online: 24 February 2016
© Springer-Verlag Berlin Heidelberg 2016

Abstract

Objective Orthodontic appliances are considered to be highly biocompatible although adverse effects attributed to the release of nickel ions (Ni^{2+}) have been documented. Self-ligating brackets have grown in popularity for economic reasons and supposed friction reduction. The aim of the present prospective cohort study was therefore to determine salivary Ni^{2+} concentrations in patients undergoing orthodontic treatment with self-ligating fixed appliances.

Materials and methods A group of 30 patients between 10 and 13 years of age were treated with self-ligating brackets (SmartClipTM), molar bands, and nickel–titanium (NiTi) archwires. Unstimulated saliva samples were collected after different time points (before treatment, after self-ligating bracket and band placement, before archwire insertion, after archwire insertion, and finally 4 and 8 weeks afterwards) and analyzed with an ICP mass

spectrometer followed by generalized estimating equation modelling with $\alpha = 5\%$.

Results The baseline median salivary Ni^{2+} concentration was $21.85\ \mu\text{g/l}$, while the Ni^{2+} concentrations at the following visits ranged between 13.73 and $85.34\ \mu\text{g/l}$. Significant increases in Ni^{2+} levels compared to the baseline levels were detected after band/bracket placement [$+59.76\ \mu\text{g/l}$; 95 % confidence interval (CI) 44.88 – $74.64\ \mu\text{g/l}$; $P < 0.001$] and after archwire insertion ($+53.55\ \mu\text{g/l}$; 95 % CI 25.57 – $81.52\ \mu\text{g/l}$; $P < 0.001$). After 4 weeks, Ni^{2+} concentrations returned to initial control levels or were lower.

Conclusion Self-ligating orthodontic appliances may affect salivary Ni^{2+} concentrations in vivo over the short term. However, levels resembled those documented in conjunction with conventional bracket use and remained below the daily dietary Ni intake.

Keywords Nickel release · Corrosion · Self-ligating fixed appliance · ICP mass spectrometry · Biocompatibility

Prof. Christoph Bourauel.

L. Gölz and A. C. Knickenberg contributed equally to this article.

✉ Christoph Bourauel
bourauel@uni-bonn.de

¹ Department of Orthodontics, School of Dentistry, University of Bonn, Bonn, Germany

² Department of Oral Technology, School of Dentistry, University of Bonn, Welschnonnenstr. 17, 53111 Bonn, Germany

³ Department of Prosthetic Dentistry, Preclinical Education and Materials Science, School of Dentistry, University of Bonn, Bonn, Germany

Zusammenfassung

Hintergrund und Ziel Im Allgemeinen werden kieferorthopädische Apparaturen als hoch biokompatibel eingeschätzt, obwohl verschiedene Nebenwirkungen bedingt durch die Freisetzung von Nickelionen (Ni^{2+}) dokumentiert wurden. Selbstligierende Bracketsysteme erfreuen sich zunehmender Beliebtheit aufgrund wirtschaftlicher Aspekte und propagierter Friktionsreduktion. Ziel der vorliegenden prospektiven Kohortenstudie war es daher, die Ni^{2+} -Konzentrationen im Speichel von Patienten zu bestimmen, die einer Behandlung mit selbstligierenden festsitzenden Multibracketapparaturen unterzogen wurden.

Material und Methodik Dreißig Patienten im Alter von 10–13 Jahren wurden mit selbstligierenden Brackets (SmartClip™), Molarenbändern und Nickel-Titanium-Bögen behandelt. Unstimulierte Speichelproben wurden nach unterschiedlichen Zeitpunkten (vor Behandlung, nach Insertion selbstligierender Brackets/Bänder, 2 Wochen nach und direkt vor Bogeninsertion, direkt nach Bogeninsertion, 4 und 8 Wochen nach Bogeninsertion) gesammelt und mittels ICP(“inductively coupled plasma”)-Massenspektrometer analysiert. Die Daten wurden mittels eines verallgemeinernden Schätzgleichungsmodells mit einer Signifikanzgrenze von 5 % ausgewertet.

Ergebnisse Der Medianwert der Ni^{2+} -Konzentrationen im Speichel vor der Behandlung (Referenzwert) lag bei 21,85 µg/l. In den folgenden Sitzungen lagen die Ni^{2+} -Werte zwischen 13,73 und 85,34 µg/l. Ein signifikanter Anstieg der Ni^{2+} -Konzentrationen im Vergleich zum Referenzwert wurde nach der Insertion der selbstligierenden Brackets und Bänder (+59,76 µg/l; 95 %-Konfidenzintervall, KI: 44,88–74,64 µg/l; $P < 0,001$) und nach Bogeninsertion (+53,55 µg/l; 95 %-KI: 25,57–81,52 µg/l; $P < 0,001$) ermittelt. Nach 4 Wochen waren die Ni^{2+} -Werte wieder auf dem Referenzniveau bzw. niedriger.

Schlussfolgerung Die Daten lassen die Schlussfolgerungen zu, dass selbstligierende Multibracketsysteme die Ni^{2+} -Konzentrationen im Speichel in vivo kurzfristig beeinträchtigen. Dennoch lagen die Konzentrationen in Höhe der bei Behandlung mit konventionellen Multibracketsystemen ermittelten Werte sowie unterhalb der täglichen Nickelaufnahme durch die Nahrung.

Schlüsselworte Nickelfreisetzung · Korrosion · Selbstligierendes Multibracketsystem · ICP-Massenspektrometrie · Biokompatibilität

Introduction

Nickel (Ni) is one of the main components in contemporary orthodontic appliances [1]. Its content ranges from 8 % to more than 50 % in stainless steel and nickel titanium (NiTi) alloys, respectively. Although most orthodontic materials are considered to be highly biocompatible, in vitro and in vivo studies indicate that a certain degree of intra-oral corrosion is inevitable, leading to the release of Ni ions (Ni^{2+}) [2–9]. Thereby, various factors such as temperature variations, mechanical stress induced by chewing or grinding, pH changes, bacterial accumulation, psychological stress, and to a lesser extent the actual Ni content of orthodontic materials [6, 10–12] seem to determine the degree of Ni^{2+} release.

As a consequence of metal ion leach, orthodontic appliances are suspected of inducing various side effects.

Case reports of allergic reactions during orthodontic therapy support this notion [13, 14]. Moreover, there is in vitro evidence that Ni causes cytotoxic, immunogenic, mutagenic or carcinogenic processes depending on the chemical form, concentration, duration, and route of exposure [15–19]. The most common adverse effect in orthodontics is the induction of allergic reactions, and Ni is by far the most common contact allergen [20]. The prevalence of Ni hypersensitivity in industrialized countries seems to have steadily increased, differing between male (3–8 %) and female patients (more than 30 %) [6, 20–22]. Although Ni allergy is frequently observed during skin contact, allergic reactions in the oral cavity are rare [23, 24]. In addition, there is evidence of less prevalence of Ni hypersensitivity in patients who have undergone orthodontic treatment before ear piercing [21, 25]. Interestingly, new insights in the pathomechanism of Ni hypersensitivity have revealed that a specific innate immune receptor (toll-like receptor 4) is implicated in this event [26]. Toll-like receptors are highly conserved and ubiquitously expressed receptors that sense microbial pathogens and endogenous ligands playing a pivotal role in host immune defence. In any case, Ni-containing orthodontic materials may cause allergic reactions in Ni-sensitive patients, and the use of self-ligating fixed appliances may raise Ni^{2+} levels in vivo, reinforcing the necessity of investigations in this field.

Over the last several years, self-ligating brackets rapidly permeated the orthodontic market, partly due to successful marketing and secondarily, to claims of time reduction and clinical superiority, despite the lack of evidence [27]. Self-ligating brackets are characterized by having greater volume, in general, than conventional brackets, and by displaying more irregular morphology due to the clip-connection system. Contemporary self-ligating brackets can be divided into two categories, according to the clip that holds the archwire fixed in the bracket slot: active or passive. Apart from the potential additional release of Ni^{2+} from nickel-titanium (NiTi) clips, clips also make active and constant surface proximity possible between the wire and bracket component, which may result in rising in vivo Ni^{2+} levels. However, to date only one study has investigated the ion release of self-ligating fixed appliances in vivo with few samples and employing different measurement modalities [28].

The primary aim of this study was, therefore, to determine the amount of Ni^{2+} in the saliva of patients treated with Ni-containing self-ligating fixed orthodontic appliances and NiTi archwires. We compared our results with those of a previous study applying the same experimental protocol, but with conventionally ligated fixed appliances [29]. We hypothesized that self-ligating fixed appliances would induce higher Ni^{2+} levels than conventional

appliances due to their closer clip–wire–bracket proximity and the additional Ni originating from the bracket clip.

Materials and methods

Patient and sample management

Eligible patients for this study were selected according to the following inclusion criteria: (1) no age restriction, (2) no previous orthodontic treatment, and (3) no previous allergic reaction to Ni. Excluded were (1) patients with metal restorations, (2) patients with systemic diseases or (3) smokers. A group of 30 patients fulfilled the inclusion criteria (range 10–13 years).

Orthodontic treatment protocol

This study was designed as a pragmatic trial, as no study-specific protocol was used for the treatment planning or conduct. A self-ligating fixed appliance was used in all patients following a standard protocol (see “Appendix”). A self-ligating bracket system [made of stainless steel with a passive NiTi clip, 0.56 mm (0.022 in.) slot, SmartClip™, 3M Unitek, Monrovia, CA; Fig. 1] was used in all patients with fitted stainless steel bands on all first molars (18–21 brackets and 4 bands per patient). After placement of the fixed appliance, a 0.36 mm (0.014 in.) martensitic active NiTi archwire (True Form, smile dental, Düsseldorf,

Germany) was fully engaged in all brackets of the upper and the lower dentition. Wire stops were used in all cases to prevent the archwire from sliding, while special mechanics were used in some patients [including springs for space management ($n = 7$) or bite planes ($n = 4$)]. All fixed appliance materials together with their element composition are listed in Table 1.

Sample acquisition and analysis

Our protocol for collecting the saliva samples was based on previous studies [29, 30]. Patients were instructed to avoid rinsing and eating for at least 30 min before sampling. Before the collection of saliva samples, patients were questioned whether they had eaten nuts, chocolate, legumes or other foods high in nickel content. Unstimulated saliva samples were collected from each patient in sterile 50 ml propylene tubes (Cellstar™; Greiner Bio-One, Frickenhausen, Germany) by an unblinded clinician (ACK) at six consecutive time points: T1: before orthodontic treatment, T2: immediately after placement of the self-ligating fixed appliance (i.e., self-ligating brackets/band placement without archwire), T3: 2 weeks later and before archwire insertion, T4: immediately after archwire insertion, T5: 4 weeks after archwire insertion, and T6: 8 weeks after archwire insertion (Fig. 2).

Samples were then transferred to glass tubes (VWR International GmbH, Darmstadt, Germany) and stored at $-20\text{ }^{\circ}\text{C}$. From each sample, 1 ml of saliva was placed into an open glass vessel and dried using an infrared lamp (InfraPhil HP3616; Philips, Eindhoven, The Netherlands) for 12 h. For organic matter digestion, 0.2 ml of aqua regia (3 parts hydrochloric acid to 1 part nitric acid) was added; the vessels were closed and left for 24 h. Afterwards, the solution was diluted to a volume of 3 ml (Ampuwa water, Fresenius Kabi, Bad Homburg, Germany) and filtered (FP30, 1.2CA, Schleicher & Schuell, Dassel, Germany). Ni^{2+} levels were analyzed using an inductively coupled plasma mass spectrometer (SCIEX ELAN 5000; Perkin Elmer, Wellesley, MA, USA). Each sample was examined consecutively three times, and the mean of the three measurements taken. Results were converted according to the calibration standard (‘Standard water 1’ 10090a307; Merck KGaA, Darmstadt, Germany). The detection limit of the machine was $0.1\text{ }\mu\text{g/l}$ or 1 amu [31]. Regular calibration of the instrument warranted the detection limit and high resolution of the apparatus. Reference measurements taken with Ampuwa water were subtracted from the results.

Statistical analysis

As data were not normally distributed (tested by inspecting the histograms and via the Kolmogorov–Smirnov test with



Fig. 1 Image of the self-ligating bracket system [made of stainless steel with a passive NiTi clip, 0.56 mm (0.022 in.) slot, SmartClip™, 3M Unitek, Monrovia, CA, USA] used in this study

Abb. 1 Abbildung des in der Studie verwendeten selbstligierenden Bracketsystems [bestehend aus Edelstahl und einem passivem NiTi-Clip, 0,56-mm(0.022 in.)-Slot, SmartClip™, 3M Unitek, Monrovia/CA, USA]

Tab. 1 Composition of materials used in the study (according to manufacturer)
Tab. 1 Zusammensetzung der in der Studie verwendeten Materialien (nach Herstellerangaben)

Material	Product	Content of metal elements in percent (%)															
		Ni	C	Si	Mn	Cr	P	S	Fe	Mo	W	Ti	Co	Cu	Nb + Ta	O	H
Brackets	SmartClip; 3M Unitek, Monrovia, CA	5.00–13.00	<0.07	<1.00	<1.00	17.00	<0.04	<0.03	72.00	–	–	–	–	4.00	0.30	–	–
Bands	Ormco, Glendora, CA	10.50–13.00	0.12	1.00	2.00	%	17.00–19.00	0.05	0.03	Rest	–	–	–	–	–	–	–
Martensitic active NiTi archwires	Smile Dental, Düsseldorf, Germany	55.00	≤0.50	–	–	–	–	–	–	–	Rest	–	–	–	–	≤0.50	≤0.50
NiTi open spring	Ormco, Glendora, CA	54.00–56.00	–	–	–	–	–	–	–	–	Rest	–	–	–	–	–	–
SS closed coil spring	Ormco, Glendora, CA	8.00–10.00	0.15	1.00	2.00	17.00–19.00	0.05	0.03	Rest	–	–	–	–	–	–	–	–
<i>Ni</i> nickel, <i>C</i> carbon, <i>Si</i> silicon, <i>Mn</i> manganese, <i>Cr</i> chromium, <i>P</i> phosphorus, <i>S</i> Sulfur, <i>Fe</i> iron, <i>Mo</i> molybdenum, <i>W</i> tungsten, <i>Ti</i> titanium, <i>Co</i> cobalt, <i>Cu</i> copper, <i>Nb</i> + <i>Ta</i> niobium + tantalum, <i>O</i> oxygen, <i>H</i> hydrogen, <i>SS</i> stainless steel																	

Lilliefors correction) and inhomogeneous (subjected to Levene's test), descriptive statistics included the median, range and the interquartile range ($Q1$ – $Q3$). Mixed-effects Generalized Estimating Equation (GEE) models (Poisson family) were fitted to compare the Ni^{2+} levels, with the various time points as clustering variables. Model fit was assessed with the quasi-likelihood independence model criterion statistic proposed by Pan [32]. All analyses were performed in Stata version 10 (StataCorp LP, College Station, TX, USA) with the macro *xtgee*. All P values were two-sided with a level of significance at $\alpha = 0.05$.

Results

Salivary Ni^{2+} concentrations

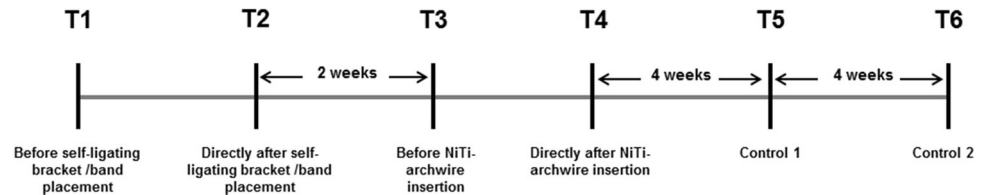
Salivary Ni^{2+} levels measured at all time points are provided in Table 2 and Fig. 3. The baseline median levels of Ni^{2+} (before placement of the self-ligating fixed appliance) were 21.85 $\mu\text{g/l}$ (reference; Table 2; Fig. 4). Median salivary Ni^{2+} concentrations ranged between 13.73 and 85.34 $\mu\text{g/l}$ according to the time points (Table 2). In detail, in comparison with baseline levels (21.85 $\mu\text{g/l}$), Ni^{2+} levels were higher after placement of the self-ligating appliance (85.34 $\mu\text{g/l}$), returned to baseline levels after 2 weeks (19.19 $\mu\text{g/l}$), rose again after placement of the NiTi archwires (57.74 $\mu\text{g/l}$) and returned again to baseline levels or lower after 4 weeks (13.73 $\mu\text{g/l}$).

Results of GEE modelling using patient and time point as the clustering variables are illustrated in Table 3. In the fitted model, the “time point” factor influenced significantly the Ni^{2+} concentrations; the predicted Ni^{2+} levels are seen in Fig. 5. Post hoc comparisons among the various time points (Table 4) revealed that significantly higher Ni^{2+} levels than the baseline levels (T1) were found after placement of the self-ligating fixed appliance (T2; difference 59.76 $\mu\text{g/l}$; 95 % CI 44.88–74.64 $\mu\text{g/l}$; $P < 0.001$) and after the insertion of the NiTi archwires (T4; difference 53.55 $\mu\text{g/l}$; 95 % CI 25.57–81.52 $\mu\text{g/l}$; $P < 0.001$). On the contrary, 4 weeks after insertion of the NiTi archwires (T5) the Ni^{2+} levels were significantly lower than the baseline levels (T1; difference –7.75 $\mu\text{g/l}$; 95 % CI –13.86 to –1.63 $\mu\text{g/l}$; $P = 0.013$), then returning to baseline levels.

Discussion

Investigations about the adverse effects of orthodontic treatment have been widely conducted in recent years, reinforced by possible implications of Ni-containing orthodontic appliances [17], the increasing prevalence of

Fig. 2 Sample management according to time points (*T*)
Abb. 2 Probenmanagement in Abhängigkeit vom Zeitpunkt der Probenentnahme (*T*)



Tab. 2 Ni^{2+} concentrations ($\mu\text{g/l}$) at each time point
Tab. 2 Ni^{2+} -Konzentrationen ($\mu\text{g/l}$) zu jedem Zeitpunkt

Time point (patients)	Median	Interquartile range	Range
T1 (<i>n</i> = 30)	21.85	15.95–27.79	12.05–56.65
T2 (<i>n</i> = 30)	85.34	52.74–113.17	18.82–172.13
T3 (<i>n</i> = 30)	19.19	17.81–25.72	11.24–143.53
T4 (<i>n</i> = 30)	57.74	25.88–91.99	7.48–343.65
T5 (<i>n</i> = 29)	13.73	10.50–20.59	4.70–49.60
T6 (<i>n</i> = 30)	19.83	16.92–24.12	8.17–42.17

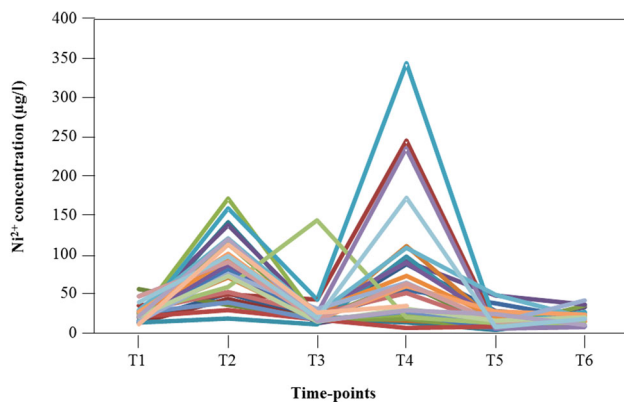


Fig. 3 Line plot of nickel concentrations in the saliva of all patients according to sample collection: *T1* reference standard; *T2* after self-ligating bracket/band placement; *T3* 2 weeks later and before wire insertion; *T4* immediately after wire insertion; *T5* 4 weeks after wire insertion; *T6* 8 weeks after wire insertion

Abb. 3 Liniendiagramm zur Darstellung der Nickelkonzentrationen im Speichel aller Patienten in Abhängigkeit vom Zeitpunkt der Probenentnahme: *T1* Referenzstandard; *T2* nach Insertion selbstligierender Brackets/Bänder; *T3* 2 Wochen nach und direkt vor Bogeninsertion; *T4* direkt nach Bogeninsertion; *T5* 4 Wochen nach Bogeninsertion; *T6* 8 Wochen nach Bogeninsertion

Ni allergy [20, 22, 33], and signs of the intraoral aging of orthodontic materials [34, 35]. Active and passive self-ligating appliances were introduced in the last decade to presumably improve sliding mechanics via the reduction of frictional forces. This was linked to enhanced tooth movement and shorter treatment durations. Although the scientific evidence to back these benefits is lacking, several orthodontists choose to use these appliances, perhaps for economic reasons [27]. In the present study we determined salivary Ni concentrations in patients treated with self-

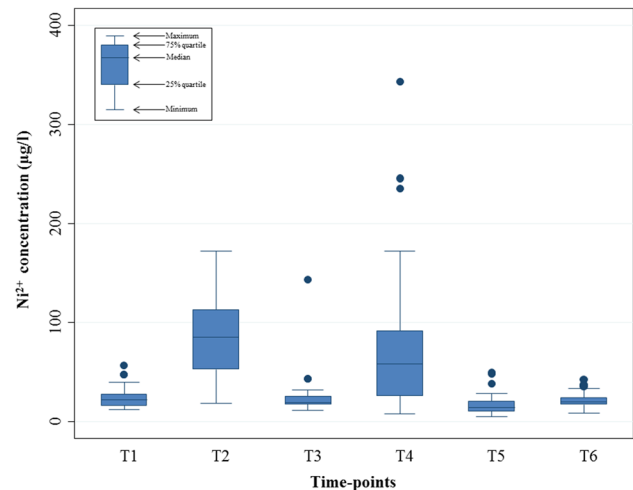


Fig. 4 Box-and-whisker diagram of nickel concentration in the saliva of all patients (*n* = 30) according to time of sample collection: *T1* reference standard; *T2* after self-ligating bracket/band placement; *T3* 2 weeks later and before wire insertion; *T4* immediately after wire insertion; *T5* 4 weeks after wire insertion; *T6* 8 weeks after wire insertion

Abb. 4 Boxplot zur Darstellung der Nickelkonzentrationen im Speichel aller Patienten (*n* = 30) in Abhängigkeit vom Zeitpunkt der Probenentnahme: *T1* Referenzstandard; *T2* nach Insertion selbstligierender Brackets/Bänder; *T3* 2 Wochen nach und direkt vor Bogeninsertion; *T4* direkt nach Bogeninsertion; *T5* 4 Wochen nach Bogeninsertion; *T6*, 8 Wochen nach Bogeninsertion

Tab. 3 Results of generalized estimating equation modelling

Tab. 3 Ergebnisse des verallgemeinernden Schätzgleichungsmodells

Factor	Coefficient	Standard error	<i>P</i> value
Time point	−7.45	0.76	<0.001
Constant	69.82	5.29	<0.001

Model information: clustering variables: patient and time point; link: identity; family: Poisson; correlation: autoregressive; Wald χ^2 = 96.70; *P* value <0.001; trace = 55.106; quasi-likelihood independence model criterion = −41,762.336

ligating fixed appliances and NiTi archwires, with Ni content ranging between 5 and 55 % (Table 1). A well-established analytic method with high detection limits concerning Ni^{2+} levels in fluids was used in accordance with previous investigations [29, 30, 36].

The present study's results are in line with those of Sahoo et al. [28], who evaluated salivary Ni^{2+} and

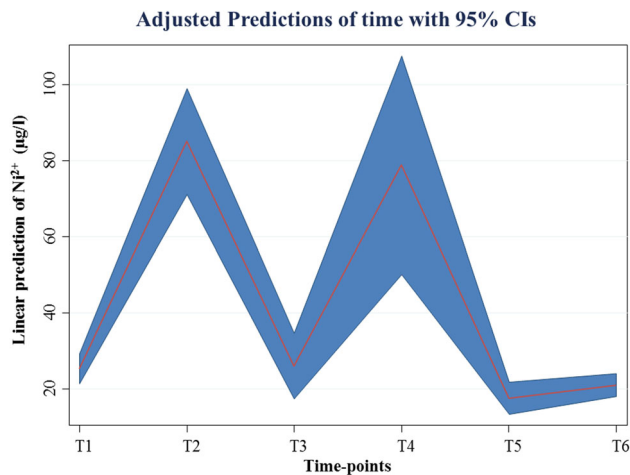


Fig. 5 Predicted marginal effects with associated 95 % confidence intervals from the generalized estimating equations model for all patients according to time of sample collection: *T1* reference standard; *T2* after self-ligating bracket/band placement; *T3* 2 weeks later and before wire insertion; *T4* immediately after wire insertion; *T5* 4 weeks after wire insertion; *T6* 8 weeks after wire insertion

Abb. 5 Prognostizierte Randeffekte bei einem Konfidenzintervall von 95 % mit dem angenommenen generalisierten Gleichstellungsmodell für alle Patienten in Abhängigkeit vom Zeitpunkt der Probenentnahme: *T1* Referenzstandard; *T2* nach Insertion selbstligierender Brackets/Bänder; *T3* 2 Wochen nach und direkt vor Bogeninsertion; *T4* direkt nach Bogeninsertion; *T5* 4 Wochen nach Bogeninsertion; *T6* 8 Wochen nach Bogeninsertion

Tab. 4 Post hoc comparisons of Ni²⁺ concentrations (µg/l) at various time points

Tab. 4 Post-hoc-Vergleiche der Ni²⁺-Konzentrationen (µg/l) zu verschiedenen Zeitpunkten

Time point	Difference (95 % CI)	<i>P</i> value
T1	Reference	
T2	59.76 (44.88, 74.64)	<0.001
T3	0.70 (−8.65, 10.05)	0.883
T4	53.55 (25.57, 81.52)	<0.001
T5	−7.75 (−13.86, −1.63)	0.013
T6	−4.30 (−10.03, 1.43)	0.142

CI confidence interval

chromium concentrations in 20 women divided in two groups. In group 1, only ten subjects were treated with conventional (MBT preadjusted edgewise brackets) fixed appliance system, and an additional ten patients received self-ligating (SmartClipTM) fixed appliance system (group 2). Both groups had 16 brackets, four bands, and 0.016 in. NiTi wires (3 M Unitec); unstimulated saliva samples were collected 1 h before as well as 1, 7, and 30 days after the appliances had been put in place [28]. Unlike in our investigation, the authors used an atomic absorption spectrometer to measure Ni²⁺ levels in patients' saliva [28]. The atomic absorption spectrometer has a higher detection

threshold and inferior resolution than inductively coupled plasma mass spectrometry [8, 29–31, 36]. Therefore, differences in methodology, sample size and time points for sample collection might explain the generally lower Ni²⁺ concentrations they documented (varying between 0.689 and 2.895 µg/l in group 1, and 0.680 and 4.950 µg/l in group 2) with self-ligating brackets. However, they detected a similar increase in Ni²⁺ after the insertion of self-ligating fixed appliances, as we did in this study. Those authors attributed their self-ligating group's higher Ni²⁺ levels compared to the conventional group 1's to the NiTi clips, a potential additional source of nickel [28].

In terms of differences between self-ligating and conventional fixed appliances, a direct comparison can be made with the study of Petoumenou et al. [29]. They used a similar protocol to assess Ni²⁺ levels in the saliva of 18 patients treated with conventional stainless steel brackets and bands (Ormco), as well as 0.016 in. NiTi archwires in both jaws, and reported baseline median Ni²⁺ concentrations of 34 µg/l (before appliance placement) that increased significantly after placement of the fixed appliance (78 µg/l) and after the archwires were inserted (56 µg/l) [29]. Our study results are consistent with the investigation of Petoumenou et al. [29], although their data were more widely dispersed than ours, due perhaps to sample or appliance differences. Large variations in measured Ni²⁺ levels in orthodontic patients were also reported by Sahoo et al. [28] and Matos de Souza et al. [37], who evaluated 30 patients with orthodontic appliances using inductively coupled plasma mass spectrometry. They attributed this variation to the effect of the dietary intake of Ni²⁺ or measurements at different time points within a day [37]. Mechanical stress induced by chewing or grinding, changes in pH, microbial oral flora and psychological stress might also influence intraoral Ni²⁺ release [6, 10–12, 38]. Finally, frictional forces between the arch and the clips, as well as the higher profile of self-ligating brackets might encourage intraoral metal leaching.

In vitro analysis of several self-ligating brackets revealed Ni²⁺ levels between 0.01 and 5.24 µg/day in a single measurement, with SmartClipTM brackets belonging to the group with the lowest Ni²⁺ levels [8]. Their data were confirmed by a recent in vitro study [39]. However, they detected higher nickel levels in the self-ligating group with SmartClipsTM compared to the conventional brackets from the same manufacturer (0.11 versus 0.00 ppm) [39]. Surprisingly, SmartClipsTM revealed the lowest nickel release after the given conventional bracket group, even lower than that associated with other conventional bracket systems from various manufacturers. In any case, the authors concluded that the aging process did not differ between groups [39]. However, one must bear in mind that these studies were performed without archwires and cannot

be extrapolated to the clinic, as the absence of friction between brackets and archwires can lead to reduced Ni^{2+} levels.

Concerning the duration of exposure, some studies report that metal leaching processes might be time dependent [11, 40]. High metal ion levels are detected 1–2 weeks after exposure to metal appliances, and then revert to their initial levels [2, 5, 11, 29], thereby supporting the hypothesis of the formation of a passivation layer that reduces the release of ions. We observed an initial increase in Ni^{2+} levels followed by normalization after 2 weeks in this study as well, as did Sahoo et al. (Fig. 3) [28].

Finally, adverse effects of orthodontic appliances can also include cytotoxic, inflammatory or allergic processes due to the release of other metal ions like cobalt, chromium, or copper [6, 10, 20, 41–43]. Hence, side effects associated with orthodontic appliances might not always be the result of Ni-induced mechanisms via toll-like receptor 4 activation. However, Ni is the main sensitizer for contact hypersensitivity and is therefore often blamed for adverse reactions during orthodontic therapy. On the other hand, a current meta-analysis demonstrated that orthodontic treatment may even reduce the incidence of Ni hypersensitivity when the treatment took place prior to Ni sensitization triggered by ear piercing [21]. Cases of Ni-induced allergic reactions during orthodontic therapy are rare and often related to extraoral appliances, as dermal Ni contact appears to induce hypersensitivity more readily than does the contact with oral mucosa. This might be associated with the functional specificity of dendritic cells, which are the main antigen-presenting cells and play a crucial role in the initial phase of contact hypersensitivity induction [44]. Novak et al. [44] showed that oral dendritic cells possess greater tolerogenicity, whereas dermal dendritic cells are characterized primarily by their pro-allergic properties that support the development of Ni allergy. Small and frequent oral Ni^{2+} concentrations during orthodontic treatment before Ni sensitization may therefore favor the induction of oral tolerance. The Ni^{2+} levels we detected were below the generally accepted daily oral dietary intake of 200–600 μg , in line with previous studies analyzing conventional fixed appliance system [45–47].

Strengths and limitations

The strengths of this study include (1) its in vivo nature, which better reflects the clinical reality than do in vitro studies on ion leaching from self-ligating fixed appliances [34], and (2) the use of a standardized protocol for the collection and analysis of the saliva samples. As we

applied no specific inclusion criteria during patient recruitment, our study results can probably be extrapolated to the average orthodontic patient treated with metallic self-ligating fixed appliances fitted on the upper and lower teeth. Limitations of this study include possible confounding in the measurement of Ni^{2+} from dietary or other sources and the fact that Ni^{2+} levels are not directly linked to adverse biological reactions to nickel.

Conclusion

Median salivary Ni^{2+} concentrations in patients with self-ligating fixed appliances ranged between 13.73 and 85.34 $\mu\text{g/l}$, remaining below the daily dietary nickel intake. The highest levels of Ni^{2+} were measured after the placement of the self-ligating brackets and bands and after NiTi archwire insertion; Ni^{2+} levels returned to baseline levels after a 2-week period. Finally, the Ni^{2+} levels detected in this study resemble those documented in studies using conventional fixed appliances discounting our hypothesis. Small amounts of nickel ions released by orthodontic materials might support the concept of an orally induced tolerance against nickel during early orthodontic treatment.

Compliance with ethical standards

Conflict of interest Lina Gözl, Anna Christine Knickenberg, Ludger Keilig, Susanne Reimann, Spyridon N. Papageorgiou, Andreas Jäger, and Christoph Bourauel state that there are no conflicts of interest.

Appendix: Protocol for the placement of the fixed orthodontic appliances

Teeth were polished with pumice and rubber cup to remove pellicle and food debris and then rinsed with water and air dried. Preformed stainless steel bands (Ormco, Glendora, CA, USA) were fitted to the patients' upper and lower first molars and cemented with glass-ionomer cement (Ketac Cem mu, 3 M ESPE, Seefeld, Germany). The buccal surfaces of the remaining teeth underwent acid etching (phosphoric acid 37 %, Ormco) for 15 s, rinsed again with water, and air dried. Monomer primer (bonding; Transbond XT, 3M Unitek, Monrovia, CA, USA) was applied on etched surfaces of the teeth followed by application of light cure composite resin (Transbond XT, 3M Unitek) on the bracket bases. Brackets (SmartClipTM, 3M Unitek) were pressed lightly onto the tooth surface and material excess removed. Polymerization was done with a halogen lamp for 30 s per tooth.

References

- Andreasen GF, Hilleman TB (1971) An evaluation of 55 cobalt substituted Nitinol wire for use in orthodontics. *J Am Dent Assoc* 82:1373–1375
- Ağaoğlu G, Arun T, İzgü B et al (2001) Nickel and chromium levels in the saliva and serum of patients with fixed orthodontic appliances. *Angle Orthod* 71:375–379
- Eliades T, Trapalis C, Eliades G et al (2003) Salivary metal levels of orthodontic patients: a novel methodological and analytical approach. *Eur J Orthod* 25:103–106
- Fors R, Persson M (2006) Nickel in dental plaque and saliva in patients with and without orthodontic appliances. *Eur J Orthod* 28:292–297
- Kerosuo H, Moe G, Hensten-Pettersen A (1997) Salivary nickel and chromium in subjects with different types of fixed orthodontic appliances. *Am J Orthod Dentofac Orthop* 111:595–598
- Kerosuo H, Moe G, Kleven E (1995) In vitro release of nickel and chromium from different types of simulated orthodontic appliances. *Angle Orthod* 65:111–116
- Kocadereli I, Ataç A, Kale S et al (2000) Salivary nickel and chromium in patients with fixed orthodontic appliances. *Angle Orthod* 70:431–434
- Luft S, Keilig L, Jäger A et al (2009) In-vitro evaluation of the corrosion behavior of orthodontic brackets. *Orthod Craniofac Res* 12:43–51
- Amini F, Rahimi H, Morad G et al (2013) The effect of stress on salivary metal ion content in orthodontic patients. *Biol Trace Elem Res* 155:339–343
- Barrett RD, Bishara SE, Quinn JK (1993) Biodegradation of orthodontic appliances. Part I. Biodegradation of nickel and chromium in vitro. *Am J Orthod Dentofac Orthop* 103:8–14
- Huang HH, Chiu YH, Lee TH et al (2003) Ion release from NiTi orthodontic wires in artificial saliva with various acidities. *Biomaterials* 20:3585–3592
- Ehrnrooth M, Kerosuo H (2009) Face and neck dermatitis from a stainless steel orthodontic appliance. *Angle Orthod* 79:1194–1196
- Kolokitha OE, Chatzistavrou E (2009) A severe reaction to Ni-containing orthodontic appliances. *Angle Orthod* 79:186–192
- Costa M, Zhuang Z, Huang X et al (1994) Molecular mechanisms of nickel carcinogenesis. *Sci Total Environ* 148:191–199
- Fernández-Miñano E, Ortiz C, Vicente A et al (2011) Metallic ion content and damage to the DNA in oral mucosa cells of children with fixed orthodontic appliances. *Biomaterials* 24:935–941
- Gözl L, Bayer S, Ludger K et al (2014) Possible implications of Ni(II) on oral IL-1 β -induced inflammatory processes. *Dent Mater* 30:1325–1335
- Natarajan M, Padmanabhan S, Chitharanjan A et al (2011) Evaluation of the genotoxic effects of fixed appliances on oral mucosal cells and the relationship to nickel and chromium concentrations: an in vivo study. *Am J Orthod Dentofac Orthop* 140:383–388
- Wataha JC, Lockwood PE, Marek M et al (1999) Ability of Ni-containing biomedical alloys to activate monocytes and endothelial cells in vitro. *J Biomed Mater Res* 45:251–257
- Simonsen AB, Deleuran M, Johansen JD et al (2011) Contact allergy and allergic contact dermatitis in children—a review of current data. *Contact Dermat* 65:254–265
- Gözl L, Papageorgiou SN, Jäger A (2015) Nickel hypersensitivity and orthodontic treatment: a systematic review and meta-analysis. *Contact Dermat* 73:1–4
- Rietschel RL, Fowler JF, Warshaw EM et al (2008) Detection of nickel sensitivity has increased in North American patch-test patients. *Dermatitis* 19:16–19
- Bass JK, Fine H, Cisneros GJ (1993) Nickel hypersensitivity in the orthodontic patient. *Am J Orthod Dentofac Orthop* 103:280–285
- Schuster G, Reichle R, Bauer RR et al (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. *J Orofac Orthop* 65:48–59
- Fors R, Persson M, Bergström E et al (2012) Lifestyle and nickel allergy in a Swedish adolescent population: effects of piercing, tattooing and orthodontic appliances. *Acta Derm Venereol* 92:664–668
- Schmidt M, Raghavan B, Müller V et al (2010) Crucial role for human toll-like receptor 4 in the development of contact allergy to nickel. *Nat Immunol* 11:814–819
- Papageorgiou SN, Konstantinidis I, Papadopoulou K, Jäger A, Bourauel C (2014) Clinical effects of pre-adjusted edgewise orthodontic brackets: a systematic review and meta-analysis. *Eur J Orthod* 36:350–363
- Sahoo N, Kailasam V, Padmanabhan S, Chitharanjan AB (2011) In-vivo evaluation of salivary nickel and chromium levels in conventional and self-ligating brackets. *Am J Orthod Dentofac Orthop* 140:340–345
- Petoumenou E, Arndt M, Keilig L et al (2009) Nickel concentration in the saliva of patients with nickel titanium orthodontic appliances. *Am J Orthod Dentofac Orthop* 135:59–65
- Petoumenou E, Kislyuk M, Hoederath H et al (2008) Corrosion susceptibility and nickel release of nickel titanium wires during clinical application. *J Orofac Orthop* 69:411–423
- Selby M, Hieftje GM (1987) Inductively coupled plasma-mass spectrometry: a status report. *Am Lab* 19/16:16–28/28–40
- Pan W (2001) Akaike's information criterion in generalized estimating equations. *Biometrics* 57:120–125
- Hogeling M, Pratt M (2008) Allergic contact dermatitis in children: the Ottawa hospital patch-testing clinic experience, 1996 to 2006. *Dermatitis* 19:86–89
- Eliades T, Bourauel C (2005) Intraoral aging of orthodontic materials: the picture we miss and its clinical relevance. *Am J Orthod Dentofac Orthop* 127:403–412
- Pandis N, Bourauel C, Eliades T (2007) Changes in the stiffness of the ligating mechanism in retrieved active self-ligating brackets. *Am J Orthod Dentofac Orthop* 132:834–837
- Arndt M, Brück A, Scully T et al (2005) Nickel ion release from orthodontic NiTi wires under simulation of realistic in situ conditions. *J Mater Sci* 40:3659–3667
- Matos de Souza R, Macedo de Menezes L (2008) Nickel, chromium and iron levels in the saliva of patients with simulated fixed orthodontic appliances. *Angle Orthod* 78:345–350
- Papadopoulou K, Eliades T (2009) Microbiologically-influenced corrosion of orthodontic alloys: a review of proposed mechanisms and effects. *Aust Orthod J* 25:63–75
- Maia LH, Lopes Filho H, Ruellas AC, Araújo MT, Vaitsman DS (2014) Corrosion behavior of self-ligating and conventional metal brackets. *Dental Press J Orthod* 19:108–114
- Park HY, Shearer TR (1983) In vitro release of nickel and chromium from simulated orthodontic appliances. *Am J Orthod Dentofac Orthop* 84:156–159
- Amini F, Jafari A, Amini P et al (2012) Metal ion release from fixed orthodontic appliances—an in vivo study. *Eur J Orthod* 34:126–130
- Issa Y, Brunton P, Waters CM et al (2008) Cytotoxicity of metal ions to human oligodendroglial cells and human gingival fibroblasts assessed by mitochondrial dehydrogenase activity. *Dent Mater* 24:281–287
- Schmalz G, Garhammer P (2002) Biological interactions of dental cast alloys with oral tissues. *Dent Mater* 18:396–406
- Novak N, Gros E, Bieber T et al (2010) Human skin and oral mucosal dendritic cells as 'good guys' and 'bad guys' in allergic immune responses. *Clin Exp Immunol* 161:28–33

44. Dabeka RW, McKenzie AD (1995) Survey of lead, cadmium, fluoride, nickel, and cobalt in food composites and estimation of dietary intakes of these elements by Canadians in 1986–1988. *J AOAC Int* 78:897–909
45. Nielsen GD, Flyvholm M (1984) Risks of high nickel intake with diet. *IARC Sci Publ* 53:333–338
46. Staffolani N, Damiani F, Lilli C et al (1999) Ion release from orthodontic appliances. *J Dent* 27:449–454
47. Gjerdet NR, Erichsen ES, Remlo HE et al (1991) Nickel and iron in saliva of patients with fixed orthodontic appliances. *Acta Odontol Scand* 49:73–78