Original Article Quantification of metal trace elements in orthodontic polymeric aligners and retainers by inductively coupled plasma mass spectrometry (ICP-MS)

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Abstract: The aim of this study was to assess the metal elements in two commonly used orthodontic polymeric aligner and retainer, and evaluate the *in vivo* accumulation in blood by animal experimentation. Elements of Al, Ti, V, Cr, Fe, Co, Ni, Cu, Zn, Sn were quantified in appliances before-use and after two weeks' wearing. Intraoral usages were modeled in Wistar rats and vein blood was extracted at Day 28, Day 56 and Day 112. Trace elements analyses were conducted by inductively coupled plasma mass spectrometry (ICP-MS). Al, Ti, V, Cr, Fe, Co, Ni, Cu, Zn were detected in retainers, and Al, Ni, Zn and Sn were found in aligners. The identified element levels were significantly decreased after two weeks' intraorally wearing. The contents of metal in polymeric materials neither exceed the toxic levels nor the daily dietary intake. No *in vivo* accumulation effects were found in blood.

Keywords: ICP-MS, orthodontic polymeric material, trace element, blood sample

Introduction

Invisible orthodontic materials are finding increasing popularity because of the growing consciousness for aesthetics. With excellent transparency and formability, polymeric thermoplastic appliances have been endowed with the properties of clear, comfort, and convenience, which are extensively applied in orthodontics [1]. Polymeric aligners and retainers are two frequently-used appliances in clinical practice. Aligners usually stay in the patients' mouth for a prolonged period of one to two years and exchanged at two-week intervals. Retainers are generally located intraorally for two years or longer. As a result, the biocompatibility of thermoplastic materials has received increasing concern in recent years [2, 3]. Researches on the assessment of the biosafety of orthodontic polymeric materials were limited. Eliades et al. studied the in-vitro cytotoxic properties of invisalign appliances on human gingival fibroblasts and breast cancer cells [4]. Premaraj et al. assessed the cellular responses of oral epithelium to thermoplastic material [2]. Schuster et al. and Gracco et al. evaluated the leaching of monomers and oxidative byproducts from polymeric aligners [5, 6]. And yet, investigation of metallic levels has never been reported concerning orthodontic polymeric materials.

Cytotoxicity and sensitization are two essential component of biocompatibility. Sensitization occurs when the level of allergen achieves the immunological priming. Antigenicity for the allergic reaction is 5 to 12 times stronger in the oral mucous than that on skin. And metals are one of the most common triggers in oral allergy for the high haptenic potential [7-9]. Generally, dental allergy to metals is initiated by ions that are continuously corroded from appliances under oral conditions [10]. Factors including the friction in wearing-plucking process, enzymatic and microbial activity, the fluctuation of oral pH may aggravate the process of ionic release [7].

Essential microelements are vital to human physiological processes, whereas excessive exposures result in undesirable consequences. Toxic elements or heavy metal elements can lead to noxious effects even at low concentrations [11]. To prevent the adverse health effects



Figure 1. A. Intraoral image of the test group with plastic sheet, MSI and stainless-steel ligature. B. Intraoral image of the control group with MSI and stainless-steel ligature.

from substances in occupational and environmental settings, there is a growing need to supervise the microelements' level from anthropogenic or anomalously natural sources. For biological monitoring of heavy metal exposure in toxicology, whole blood and urine samples are the most widely used and accepted matrix to assess internal xenobiotic exposures [12].

Inductively coupled plasma mass spectrometry (ICP-MS) is an instrumental method for the investigation of trace elements for the capability of rapid multi-elements analysis of very low concentrations of elements in material, which remarkably improved the determination of the presence of metals in biological matrices [13]. The aim of this study was to quantitative assess the metal elements in two commonly used orthodontic polymeric aligner and retainer, and evaluate the in vivo accumulation effects in blood by animal experimentation.

Materials and methods

Clinical polymeric samples collection

A polymeric aligner and a polymeric retainer were investigated in our study, which were commonly used in orthodontics. 30 as-received Invisalign Smart Track aligners (Align Technology, California, US) and 30 Erkodur retainers (Erkodent Erich Kopp, Pfalzgrafenweiler, Germany) were collected to evaluate the trace element levels before-use.

The after-use samples were taken from the aligners and retainers worn intraorally for two weeks. 30 patients (10 males, 20 females; 27.07±5.48 years) treated with Invisalign Sma-

rt Track aligners and 30 patients (17 males, 13 females: 22.30±5.07 years) underwent Erkodur retainers were selected at the Capital Medical University Department of Orthodontics, respectively, who fulfilled the following criteria: absence of oral or systematic diseases, no history of previous orthodontic treatment. no oral implants or prosthetics, and nonsmoking. Patients were required to wear the aligner or retainer for 20 to 22 hours a day, and to remove them when eat or brush teeth. Warm flowing water was instructed to clean the appliances. After two weeks, the after-use aligners and retainers were collected, cleaned with a soft brush under running water and dried with compressed air.

Animal experimentation

Eight-week old male Wistar rats (n=80, bw 297.80 \pm 9.77 g) were obtained from the Animal Laboratory at the Beijing Stomatological Hospital, and housed in a temperature (23 \pm 2°C) and humidity controlled room (55 \pm 5% RH) under a 12 h light and dark cycle. Animals were kept in polycarbonate cages with stainless-steel meshed lid and floor, with food and water at libitum.

A mini-screw implant (MSI; AbsoAnchor SH12-11-05, 1.2×5 mm, Dentos, Korea) was placed in the middle palate of rats. A sheet of thermoplastic sample was fixed with MSI by a piece of stainless-steel ligature at one side, and the other side was linked with incisors (**Figure 1A**).

The mass of the intraoral thermoplastic sheet was adjusted from a pair of the mean weights

LOQ (ng/ml)	LOD (ng/ml)					
3.94	1.21					
0.682	0.224					
0.763	0.251					
0.714	0.239					
0.443	0.134					
1.368	0.428					
0.659	0.199					
1.749	0.589					
3.82	1.47					
0.397	0.131					
	LOQ (ng/ml) 3.94 0.682 0.763 0.714 0.443 1.368 0.659 1.749 3.82 0.397					

Table 1. Limits of quantification (LOQs) anddetection (LODs)

of aligners or retainers based on the ratio of human and rat body weights, which was defined as the routine mass (RM). 50 respective unused aligners and retainers were applied to calculate the mean weights of the two (Data not show, aligner: 2.66 ± 1.76 g/per pair, retainer: $3.22\pm$ 0.92 g/per pair). The average human body weight was taken as 60 kg. To assess the extreme elemental accumulation in blood, a routine mass group (RM group) and a triple mass group (TM group) were used in this study, because a greater mass of thermoplastic sheet was inapposite for the intraoral fixation in the rat. The sheets were replaced every week according to the rat body weights.

Routine mass = the weights of average aligners or retainers × rat body weigh/average human body weight (60,000 g).

Triple mass = $3 \times \text{Routine mass}$.

Rats were divided into four groups as test group of aligner (n=30, bw 299.85±10.21 g), test group of retainer (n=30, bw 295.57±9.64 g), control group (n=10, bw 299.20±8.55 g) and blank group (n=10, bw 296.95±9.84 g). The two test groups were further equally divided into routine mass and triple mass groups. Rats in control group were fixed the same MSI and stainless-steel ligatures without thermoplastic sheets (**Figure 1B**). 0.5 mL blood samples were obtained from the rat orbital vein of each object at Day 28 (D28), Day 56 (D56) and Day 112 (D112). Samples were filled into a lithium heparin vacuum tube and stored at -80°C.

Ethical consideration

Ethical approval was obtained from the Ethical Committee of Capital Medical University for the aim of this study. Written consents were taken from the subjects or their patients after detailed explanation. Animal experimentations followed the ethical guidelines established by the Ministry Office of Experimental Animals of Capital Medical University and in accordance with the Guide for the Safety and Welfare of Laboratory Animal of Administration Office of Laboratory Animal, Beijing, China.

Multi-element analysis

0.5 g thermoplastic sample or 0.5 mL whole blood sample was accurately weighted into a digestion vessel. 1.5 mL 65% nitric acid and 0.5 mL 30% H_2O_2 were added to each sample. A microwave digestion system (LabTech, US) was used. The temperature was increased in a four-step program up to 180°C in 15 minutes. Digestion conditions for microwave system were applied as 5 minutes for 1800W at 100°C, 10 minutes for 1800 W at 150°C, 15 minutes for 1800 W at 180°C, and kept at 180°C for 10 minutes. After cooling to room temperature, the sample solution was quantitatively transferred to a plastic container and diluted to 25 mL with deionized water. Concentration of objective elements was determined by ICP-MS (Agilent 7700 serires, Agilent Technologies, Japan). The instrumental operating conditions were set as follows: RF power 1550 W, auxiliary gas flow rate 0.9 L/min, nebuliazer gas flow rate 1.02 L/ min, nebuliazer pump 6 rmp, sampling depth 6 mm, spray chamber temperature 2°C, respectively. Bismuth and rhodium were used as the internal standard elements. Ten elements have been quantified: aluminium (Al), titanium (Ti), vanadium (V), chromium (Cr), ferrum (Fe), cobalt (Co), nickel (Ni), cuprum (Cu), zinc (Zn), tin (Sn).

The limits of detection (LODs) and quantification (LOQs) for the measurements were determined from the blank solutions prepared as the samples, separately from the blank and calibration standards (Inorganic Venture, US). The ten blank solutions used for LODs and LOQs calculations were measured on different days. The LODs and LOQs were calculated from three times and ten times the standard deviation of the blank concentration, respectively. The concentrations of any elements whose values were lower than the detection limit were considered to be 0 (**Table 1**).

Statistical analysis

Statistical analyses were performed using the Statistical Package for the Social Sciences v22

	Before-use; n=30; mg/kg				After-use; n=30; mg/kg				
	Mean ± SD	5^{th}	95 th	DR	Mean ± SD	5^{th}	95 th	DR	F value
AI	46.94±18.16	40.16	53.73	100%	0.00561±0.00331	0.00438	0.00685	86.7%	< 0.001
Ti	4.48±1.59	3.88	5.07	100%	0.58992±0.63020	0.35460	0.82523	100%	< 0.001
Fe	5.04±2.05	4.28	5.81	100%	0.00233±0.00163	0.00172	0.00294	80%	< 0.001
V	0.01446±0.00771	0.01157	0.01734	100%	0.00035±0.00043	0.00018	0.00051	40%	< 0.001
Со	8.55±3.51	7.24	9.86	100%	0.60496±0.46099	0.43283	0.77710	100%	< 0.001
Ni	0.13±0.43	0.12	0.15	100%	0.0156±0.0135	0.01051	0.02059	76.7%	< 0.001
Cr	5.25±1.75	4.59	5.90	100%	0.00015±0.00035	0.00002	0.00028	16.7%	< 0.001
Cu	0.80±0.11	0.75	0.84	100%	0.10241±0.05634	0.08137	0.12345	100%	< 0.001
Zn	4.88±2.21	4.06	5.71	100%	0.82769±0.53752	0.62697	1.02840	100%	< 0.001
Sn	0	0	0	0%	0	0	0	0%	1.00

Table 2. Elemental quantification for retainers

SD, standard deviation; DR, detected rate.

Table 3. Elemental	quantification	for aligners
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	Before-use; n=30; µg/kg				After-use; n=30; µg/kg				Dvoluo
	Mean ± SD	5^{th}	95 th	DR	Mean ± SD	5^{th}	95 th	DR	Pvalue
AI	21.24±8.14	18.19	24.283	100%	1.60±3.15	0.43	2.78	23.6%	<0.001
Ti	0	0	0	0%	0	0	0	0%	1.00
Fe	0	0	0	0%	0	0	0	0%	1.00
V	0	0	0	0%	0	0	0	0%	1.00
Со	0	0	0	0%	0	0	0	0%	1.00
Ni	60.27±26.94	50.21	70.33	100%	15.99±5.70	13.87	18.12	100%	<0.001
Cr	0	0	0	0%	0	0	0	0%	<0.001
Cu	0.34±1.10	-0.07	0.75	10%	0.15±0.57	-0.06	0.36	7%	0.385
Zn	152.12±84.30	120.64	183.60	100%	78.97±26.29	69.15	88.79	100%	<0.001
Sn	30144±1708.6	29506	30782	100%	16397±3097.5	15240	17553	100%	<0.001

SD, standard deviation; DR, detected rate.

(SPSS; SPSS Inc, Chicago, USA). Normality of distribution of the data was assessed by Kolmogorov-Smirnov test. Descriptive data were reported as mean ± standard deviation (SD), and 5-95th percentiles. Elemental changes before and after-use were assessed by Paired t-test and Wilcoxon test with abnormal distribution. Kruskal-Wallis test and Dunnett-t post hoc test was applied to evaluate the differences of elemental amounts in blood samples at each time interval. Friedman test was used to estimate the differences of elemental changes in three time points. Differences were considered significant when P<0.05.

Results

In the case of retainer samples before-use, Measurable Al, Ti, Fe, V, Co, Ni, Cr, Cu, Zn and Sn were observed. The highest mean level was found in AI ($46.94\pm18.16 \text{ mg/kg}$), followed by Co ($8.55\pm3.51 \text{ mg/kg}$) and Cr ($5.25\pm1.75 \text{ mg/kg}$). None of the data were normally distributed. The level of all the observed elements was decreased significantly after two weeks' wearing (P<0.001). Measurable Ti, Co, Cu and Zn were quantified in all samples, and low levels of AI, Fe, V, Ni and Cr, were identified with detectable rates of 86.7%, 80%, 40% 76.7% and 16.7% respectively. Sn was not detected in both before and after use samples assayed (**Table 2**).

Measurable Al (21.24 \pm 8.14 µg/kg), Ni (60.27 \pm 26.94 µg/kg), Zn (152.12 \pm 84.30 µg/kg) and Sn (30.14 \pm 1.71 mg/kg) were detected in aligner samples before-use. Cu was only found in 10% samples. Low level of Al was detected in 23.6% samples worn intraorally for two weeks. Ni, Zn and Sn were detected with significant decrease in contents. No significant differences were found regarding Cu between samples before and after use (P=0.385). None of the data were normally distributed. Ti, Fe, V, Co and Cr were found to be below the detection limits in both before and after use samples assayed (**Table 3**).

In the case of blood sample, AI, Ti, V, Cr, Fe, Co, Ni, Cu, Zn and Sn were observed in all groups. **Figure 2A-J** showed the data distribution (mean and SD) of investigated elements. The ion levels presented no difference among the three time points (data not show). Statistically significant differences were only found regarding in the mean levels of Ti and Fe between blank group and others (**Figure 2B** and **2C**; P<0.001).

Discussion

The biocompatibility of fixed orthodontic appliances have been extensively studied in terms of examining ionic release and toxic effect related to cytotoxicity and genotoxicity [3]. And yet, few investigations have been made on the issue concerning metallic elements from polymeric materials. One harmfulness of the intraoral release of metal ions is the noxious effect to cells of the adjacent tissues, which may cause pathological and clinical responses including cytotoxicity, allergies, and mutagenesis [14]. Harmfulness is the accumulation effect. Chronic exposure to trace metals result in bioaccumulation and biomagnified into body tissues, causing potential health risks like disruption of the central nervous system, reproductive failure, genotoxicity, and gastro-intestinal problems [15].

According to the material safety data sheet (MSDS), the chemical composition was PETG (polyethylene terephthalate glycol) for the retainer and TPU (thermoplastic polyurethane) for the aligner, but no information of metallic levels was provided [16, 17]. Though not serve as the main components, metals are ubiquitous in the course of production and processing. Moreover, metallic ions can alter cellular metabolism and morphology even at extremely low concentration, and chronically expose to low levels of metal ions can produce inflammation and even DNA instability. Therefore, monitoring the precise amounts of these ions is still a noteworthiness subject [18]. To our knowledge, this was the first time that levels of metals were reported in terms of orthodontic polymeric materials.

Aluminum is a known toxic element at high doses, related to the occurrence of neurodegenerative disorder, pulmonary disease, metabolic bone disease and allergic effect [19]. A. Martín-Cameán et al. have first evaluated the release of aluminum in fixed orthodontic appliances [20]. The content and variation of aluminum were both the greatest in polymeric retainers, with an average level of 46.94 mg/kg. However, when it is applied to retainers' weight, it is about 0.227 mg per pair. Seeing that the maximum ion release always occurred after the first day [9], extreme hypothesis could be made that all aluminum was leached at one day. In the adult population, daily mean exposure to aluminum varied from 3 to 1000 mg according to different ethnicity and dietary habit. The Joint Food and Agriculture Organization/World Health Organization Expert Committee on Food Additives (JFAO/WHO) have assessed the provisional tolerable weekly intake (PTWI) at 2 mg Al/kg bw [21]. The aluminum concentrations in most unprocessed foods is less than 5 mg/kg, while in processed foods, the contents are several or even hundreds times higher [19]. Thus, compared with daily dietary intake, investigated polymeric appliances meet the biosafety requirements for aluminum.

Titanium and iron are common compositional elements in fixed orthodontic appliances. As the main content of stainless steel materials, significant releases of Fe were reported in different extents [20, 22]. Animal experimentation indicated that the continually elevated Fe level in test groups might come from the stainlesssteal ligatures (0Cr18Ni9), which were simultaneously exchanged alone with the thermoplastic sheets every week. Titanium is the main constituent of the alloy producing archwires and implants. Literatures presented different results in Ti release according to the type of alloys. Negative or very low releases were reported in TMA and Ni, Ti arch wires probably due to the high corrosion resistant property [22, 23]. Others found the leaching out of Ti at different degrees from mini-implants [13, 24]. Considering the MSI used in our study were made of titanium-aluminum-vanadium alloy (Ti6Al4V), the raised titanium level in animal blood samples accorded with the point that,



despite the passive activity of external layer of oxides and great biocompatibility, electrochemical and galvanic erosion may take place in the environment of oral cavity in terms of titanium implant [25].

Vanadium accounts for 3.5-4.5% of Ti6AI4V alloy. Morais found the accumulation effects of V in organs of rabbits with Ti6Al4V miniimplant [26]. Others showed no difference in blood and urine between patients with and without implants [13]. The vanadium level in retainers was comparable to the concentrations in food ranging 1 to 30 μ g/kg [27]. Cobalt was presented in stainless steel appliances. Previous research on oral mucosa cell of patients underwent orthodontics found that the Co release was negligible [20, 28]. The mean cobalt content in retainers (27.5 µg per pair) was acceptable according to the daily intake from food and beverages (5-50 µg/day) [29].

In connection with the type IV delayed hypersensitivity response, nickel is the metal that causes most number of immunologic reactions with a prevalence of up to 28% depending on gender and ethnicity [7]. In vivo and in vitro researches demonstrated nickel leaching varies from 0.03 μ g/L to 170 μ g/L depends on the alloy type, manipulation of appliances, and chemical and physical conditions [7, 23, 30]. The release rate has no strict correlation with nickel content, whereas related closely to the composition and corrosion resistance of materials [31]. Though many orthodontic appliances contain nickel, existing evidences couldn't conclude that orthodontic treatments increase the induction of nickel hypersensitivity [32]. According to a vitro study [33], nickel would stimulate innate immune response and promote inflammatory in a time-depending manner and relatively high concentration (4000 ng/mL), compared with clinical nickel level (400 ng/ mL), which greatly exceeded the nickel content in polymeric appliances.

Chromium is another strong sensitizer of metalinduced allergic contact dermatitis. Controversial results existed regarding the release and accumulation of chromium from orthodontic appliances. Mikulewicz *et al.* found that Cr concentration was increased to 101 μ g/L in the saliva of patients undergoing fixed orthodontics compared with that in the control (8.18 μ g/L)

[34]. Amini et al. noted the Cr saliva level increased after orthodontic treatment [35]. Hafez et al found the Cr cellular content increase from 0.31 ng/ml to 0.78 ng/ml after 6 months' orthodontic treatment, which was inconsistent with the finds of A. Martin-Camean et al, who found the increase was negligible [8, 20]. Assessments of the daily Cr release during fixed orthodontic treatment ranged from 0.195 µg to 0.3 µg [23, 36], which is very small compared with daily dietary intake [37]. The stable forms of Cr are the trivalent and hexavalent species. Trivalent Cr. found in foods and dietary supplement, is one of the safest nutrients considering the ratio of amount needed to that can be consumed without side effect. Whereas hexavalent Cr, mostly prevailed in industrial materials, is several fold more toxic [38]. Studies presented inconsistent results in terms of valence states of ionic Cr release [25]. Since the knowledge of chemical and physical states of corrosion products is limited, the optimistic assessment can only be considered as rough guidelines at best [39]. For the level was undetectable in most samples after two weeks' wearing, we can extremely hypothesis that all chromium was released into oral cavity in one day with average amount of 16.8 µg in a pair of polymeric retainers, which is still guite below the recommended daily dose (RDD) at 35-120 µg/day [37].

Copper and zinc are essential micronutrients with extensive biofunctions, whereas potential toxicants when reaching high concentrations. Releases of copper were reported from stainless steel and NiTi alloy appliances in vitro and in vivo [9, 23, 28, 36, 40]. In relation to zinc, Mikulewicz et al. identified significant increase from fixed orthodontics in artificial saliva [34]. Goncaves et al. determined the zinc release in orthodontic bands with silver solder [40]. Though the two metals are not the main components of most dental alloys, their contents are strictly controlled for the high cytotoxicity and susceptibility to corrosion [41]. The net release of the two metals were remarkably lower than the release from fixed orthodontic appliances in literatures, as well as below the toxic levels and the daily dietary intakes [9].

In our investigation, tin was the element only presented in aligners instead of retainers. Though tin is not an essential nutrient for human, it serves as a necessary element for the growth in rats. Metallic tin is generally considered of low oral toxicity due to their poor alimentary absorption [42]. Organic tin compounds are more toxic than inorganic tin, which may cause skin irritation, systemic and neurological damages. According to JFAO/WHO, the maximum tolerable daily intake for tin is 2 mg/ kg bw, which is equivalent to 12.0 mg per day for a 60 kg adult [43]. Release of tin from orthodontic appliances has hardly been reported. Though ranked highest among investigated elements in aligner material, the content of tin is still very small compared with daily intake.

The metallic trace elements might come from the manufacturer's process or additives. Though there were no effects of toxicity and sensitization provided in MSDS of products, anecdotal reports in terms of adverse reactions to thermoplastic materials existed on papers, blogs and websites [2, 44]. The amount of ions released neither exceed the toxic levels nor the daily dietary intake; nevertheless, attentions should be paid to the presence of metal elements since sensitive individuals may react to allergen even at minute trace levels. No accumulation effects in blood were observed in our study. This could probably attribute to the low elemental contents in material, which was indicative of the biosafety in metallic level.

In this animal experimentation, plastic sheets were placed on the rats' palatal, because the grinding habit of rodents made fixation on teeth very easy to fall off. Exchange frequency of intraoral sheet was higher than that in clinical practice for the consideration of the sample abrasions and growing animal weights, as well as compensation of the shorter observation period compared with the clinical usage period for retainers and aligners to some extent. This study cannot obviate the possible accumulation of these elements in organs and tissues. Prolonged exposures of trace elements could lead to specifically organic storage for the tissue affinity of particular elements. Further studies using other possible matrices or tissues and longer observation periods (one or two years) are required to confirm the biocompatibility of orthodontic polymeric materials.

Conclusion

Al, Ti, V, Cr, Fe, Co, Ni, Cu, Zn were detected in polymeric retainers, and Al, Ni, Zn and Sn were

found in polymeric aligners, with the amounts neither exceed the toxic levels nor the daily dietary intakes. The identified element levels were significantly decreased after two weeks' intraorally wearing. No in vivo accumulation effects by polymeric materials were found in blood. The elevated Fe and Ti levels may own to the fixation appliances of stainless-steel ligatures and MSI respectively.

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Disclosure of conflict of interest

None.

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