Cytotoxicity, genotoxicity, and metal release in patients with fixed orthodontic appliances: A longitudinal in-vivo study

Hend Salah Hafez,[a] Essam Mohamed Nassef Selim,[b] Faten Hussein Kamel Eid,[b] Wael Attia Tawfik,[c] Emad A. Al-Ashkar,[d] and Yehya Ahmed Mostafa[e]
Giza, Egypt

Introduction: Treatment with fixed orthodontic appliances in the corrosive environment of the oral cavity warrants in-vivo investigations of biocompatibility. **Methods:** Eighteen control and 28 treated subjects were evaluated longitudinally. Four combinations of brackets and archwires were tested. Buccal mucosa cell samples were collected before treatment, and 3 and 6 months after appliance placement. The cells were processed for cytotoxicity, genotoxicity, and nickel and chromium contents. **Results:** In the treatment group, buccal mucosa cell viability values were 8.1% at pretreatment, and 6.4% and 4.5% at 3 and 6 months, respectively. The composite score, a calculated DNA damage value, decreased from 125.6 to 98.8 at 6 months. Nickel cellular content increased from 0.52 to 0.68 and 0.78 ng per milliliter, and chromium increased from 0.31 to 0.41 and 0.78 ng per milliliter at 3 and 6 months, respectively. Compared with the control group, the treated subjects showed significant differences for DNA damage and chromium content at 3 months only. **Conclusions:** Fixed orthodontic appliances decreased cellular viability, induced DNA damage, and increased the nickel and chromium contents of the buccal mucosa cells. Compared to the control group, these changes were not evident at 6 months, possibly indicating tolerance for or repair of the cells and the DNA. (Am J Orthod Dentofacial Orthop 2011;140:298-308)

The use of various combinations of metal alloys for prolonged durations in orthodontic patients warrants special consideration regarding their biocompatibility. The oral cavity is a complete corrosion cell, with many factors that enhance the biodegradation of orthodontic appliances.[1] Saliva acts as an electrolyte for electron and ion conduction, and the fluctuation of pH and temperature, the enzymatic and microbial activity, and the various chemicals introduced into the oral cavity through food and drink are all corrosion conductors.[2] The inherent heterogeneity of each metal alloy and its use with other alloys, the microsurface discontinuity, the forces acting on the appliances, and the friction between wires and brackets also add to the corrosion process. The literature includes many in-vivo[3-8] and in-vitro[9-16] studies documenting the corrosion of orthodontic appliances, and the release of metal ions is indisputable. It has been reported that metal ions are taken up by the adjacent oral tissues.[17-19]

As pointed out by Wataha,[20] the corrosion of an alloy is of fundamental importance to its biocompatibility because the release of elements from the alloy is nearly always necessary for adverse biologic effects such as toxicity, allergy, mutagenicity, and carcinogenicity. Alloy corrosion provides free ions that affect the tissues around it. There is little evidence that elements released from casting alloys contribute significantly to systemic toxicity. The cause of this might be explained by the low release of ions over time.

Metal tolerance and the amounts causing toxicity are not well understood. Metals are not biodegradable, and their sustained release might produce irreversible toxic effects from their accumulation in the tissues. Also, the increased exposure could limit the recovery time needed

[a]Researcher, Department of Orthodontics, Oral and Dental Research Division, National Research Center, Giza, Egypt.
[b]Professor, Department of Orthodontics and Dentofacial Orthopedics, Faculty of Oral and Dental Medicine, Cairo University, Giza, Egypt.
[c]Professor and head, Orthodontics and Pedodontics Department, Oral and Dental Research Division, National Research Center, Giza, Egypt.
[d]Head, Spectroscopy Department, Physics Division, National Research Center, Giza, Egypt.
[e]Professor and director, International Clinical Research Group, Department of Orthodontics and Dentofacial Orthopedics, Faculty of Oral and Dental Medicine, Cairo University, Giza, Egypt.
The authors report no commercial, proprietary, or financial interest in the products or companies described in this article.
Reprint requests to: Yehya Ahmed Mostafa, Department of Orthodontics and Dentofacial Orthopedics, Faculty of Oral and Dental Medicine, Cairo University, 52 Arab League St, Mohandesseen, Giza, Egypt; e-mail, mangoury@usa.net.
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for cellular repair. Metal toxicity is governed by multiple factors, making it difficult to truly assess the levels that produce cellular damage.[21,22]

The general belief that there is no frank concern regarding the corrosion by-products released in orthodontic patients is not actually supported.[23,24] The literature on cancer research and metal toxicology includes many reports of the dangers of various metal ions.[21,25-27] Nickel and chromium ions, which are abundant components of most orthodontic alloys, are classified as chemical carcinogens.[28] The recent insight into the cellular and molecular mechanisms of metal toxicity might cause some concern when dealing with orthodontic appliances.[25,27,29] That is due to their direct prolonged contact with the oral tissues and their corrosion, resulting in the release of various types and amounts of metal ions.

Because most research on the amounts of metal ions released from orthodontic alloys has shown that they fall below the recommended daily dietary intakes of nickel and chromium,[3,9-13,15,16] this might be a false assurance of safety, since chronic low levels of metal ions can alter cellular metabolism and morphology, and produce inflammation and even DNA instability.[30-35] In addition, some in-vivo studies reported biologic toxicity in orthodontic patients.[18,36-38]

To test the biocompatibility (cytotoxic and genotoxic effects on human tissues) of fixed orthodontic appliances, a longitudinal controlled clinical trial was set up with 3 distinct hypotheses: (1) fixed orthodontic appliances do not have a toxic effect, (2) fixed orthodontic appliances do not have an effect on the cellular metal content in buccal mucosa cell samples, and (3) there is no difference between the effects of the various materials of fixed orthodontic appliances.

MATERIAL AND METHODS

Sixty subjects were included in this study. Forty patients required fixed orthodontic treatment, and 20 subjects served as the control group. The aims of the study and the method of cell collection were explained to all subjects, and written consent to participate was obtained. Treatment was started after the institutional ethical committee approved the protocol.

Two subjects from the control group and 8 patients dropped out of the study. The causes for not completing the 6-month study period were variable. Some subjects received medications, others underwent oral or general surgeries, others terminated treatment because of discomfort from the appliances, and 4 were excluded for loose brackets. Eighteen control (8 men, 10 women) and 28 treated subjects (6 men, 22 women) completed the study. The average ages were 21 years 6 months (± 3.3 years) in the control group and 20 years 2 months (± 4.4 years) in the treatment group.

The eligibility criteria for subject selection included nonsmokers; no oral diseases, systemic diseases, oral restorations or prosthetics; clinically healthy oral mucosa; no previous orthodontic treatment; no occupational exposure to metals; not receiving any medications or supplements; no radiographic examination during the previous 6 months; and no known allergy to costume jewelry, watches, or sources of nickel and chromium. Subjects were initially screened with a questionnaire to check whether they fit the criteria of the study. They were then clinically assessed for normal oral mucosa.[39] The orthodontic patients were all treated with fixed orthodontic appliances in both arches. The appliances consisted of 4 bands (3M Unitek, Monrovia, Calif) on the first permanent molars, brackets, and 1 type of archwire material throughout the study.[20] The archwires were replaced at 6-week intervals. A total of 4 wires were used for each patient over the 6-month period. The sizes of the archwires were 0.012, 0.014, 0.016, and 0.018 in. The archwires were fixed with 0.01-in stainless steel ligature wire (Leone, Florence, Italy). The compositions of the material alloys are given in Table I.

The patients were divided into 4 groups according to the combination of brackets and archwires. The brackets used were standard stainless steel (American Orthodontics, Sheboygan, Wis) and equilibrium titanium (Dentaurum, Ispringen, Germany), and the archwire materials were stainless steel and nickel-titanium alloys (both, GAC International, Bohemia, NY). Group 1 had stainless steel brackets and stainless steel wires (StSt-StSt). Group 2 had stainless steel brackets and nickel-titanium wires (StSt-NiTi). Group 3 had titanium brackets and stainless steel wires (Ti-StSt). Group 4 had titanium brackets and nickel-titanium wires (Ti-NiTi).

Before the start of the study, all subjects were instructed to continue brushing but not to use toothpastes and mouthwashes containing fluoride or chlorhexidine because these have been reported to increase DNA damage in buccal mucosa cells.[40,41] A sampling schedule was set up that allowed all subjects to be measured over the same period of time, not consecutively, to prevent the effect of seasonal changes on the assessment of DNA.[42]

Buccal mucosa cells were evaluated before treatment (T0), and at 3 months (T1) and 6 months (T2) after appliance placement. The cells were harvested, according to the method of Nia et al[43] by gentle scraping of the internal part of the right and left cheeks with a wooden tongue depressor. Gentle scraping was required to prevent a heterogeneous cell sample.[44] Each tongue depressor was stirred in a 2-mL tube (Eppendorf,

Table I. General composition (wt %) of the alloys used in the treatment groups according to the available material safety data sheets

Material	*Fe*	*Ni*	*Cr*	*Mn*	*Mo*	*Ti*	*Co*	*Cu*	*Si*	*Al*	*P*	*S*	*C*	*O*	*H*	*N*
Equilibrium titanium bracket (Dentaurum)	≤0.2	–	–	–	–	Rest	–	–		–	–	–	≤0.06	≤0.18	≤0.13	≤0.05
Standard stainless steel bracket (American Orthodontics)	50–80	3–15	13–23	0–5	0–5	–	0–5	0–5	0–5	0–5	–	–	–	–	–	–
Sentalloy archwire (GAC)	Not available															
Stainless steel archwire (GAC)	69.5	9	18	2	–	–	0.75	–	–	–	–	–	–	–	–	–
Stainless steel bands (3M Unitek)	Not available															
Ligature wire Leowire (Leone)	Rest	6–8	16–18	≤2	–	–	–	–	≤1	–	≤0.045	≤0.03	≤0.15	–	–	–

Fe, Iron; *Ni*, nickel; *Cr*, chromium; *Mn*, manganese; *Mo*, molybdenum; *Ti*, titanium; *Co*, cobalt; *Cu*, copper; *Si*, silicon; *Al*, aluminum; *P*, phosphorous; *S*, sulfur; *C*, carbon; *O*, oxygen; *H*, hydrogen; *N*, nitrogen.

Hamburg, Germany) prefilled with 1.5 mL of ice-cold phosphate-buffered saline solution, free of calcium and magnesium ions at pH 7.4, to detach the cells. Ten strokes on each side were enough to produce adequate cell density in the suspension. The cell suspension was stored on ice in a closed isolated container and immediately transported to the laboratory as recommended by Albertini et al.[45]

Each sample was evaluated for cellular viability by using the trypan blue exclusion dye test.[46] DNA damage was assessed by using the alkaline comet assay according to the protocol of Tice and Vasquez.[47] The lysis step included an additional step of 100 μL of 1 mg per 1 mL of proteinase K for 45 minutes to enhance the lysis step as recommended by Szeto et al.[48] Unwinding was performed for 40 minutes in electrophoresis buffer with the pH above 13 to enhance the expression of minute amounts of single-strand breaks and allow the detection of alkali labile sites to ascertain that negative results were not due to the lack of expression of damage. The electrophoresis unit was set at 20 V for 40 minutes, and the level of the buffer was adjusted until 300 mA was reached. The slides were then electrophoresed in the alkali buffer at room temperature to produce DNA migration. The optimal electrophoretic conditions stretched the supercoiling of the DNA strands and produced migration in approximately 25% of the control cells on a slide. The DNA migration in the control cells provided a guide for the interpretation of comets when lack of comet tails gives false-negative results because of DNA-DNA or DNA-protein crosslinking. The same electrophoretic unit was used throughout the study. The neutralization step was repeated 3 times, leaving the buffer for 5 minutes each time, and the slides were treated with 500 μL of ice-cold absolute ethanol for 10 to 15 minutes. Drying of the gel prevented the diffusion of DNA fragments in the gel during storage. The slides were stored overnight in a light-protected, airtight container at 4°C and scored the next morning by using 50 μL of ethidium bromide (20 μg/mL). Visualization was done in a dimmed room by using an epifluorescent microscope (Leica, Wetzlar, Germany) filled with a green filter (N2.1; excitation filter, BP515-560; dichromatic mirror, 580; suppression filter, LP 590; Leica). A 40-times objective was used. The images were captured with a colored digital camera (DFC280; Leica, Solms, Germany) supplied with Twain imaging software (version 6.11) with host application program imaging (both Leica Microsystem Imaging Solutions, Ltd., Cambridge, UK). Two slides were scored per sample, by grading 50 nucleoids per slide for a total of 100 nucleoids. Only nucleoids of the same size were chosen subjectively for scoring. A grade was given to each nucleoid according to DNA fragmentation in the comet tail. The composite score was then calculated for each sample from the 2 slides. This calculates the DNA damage by multiplying the number of nucleoids in each grade of damage by its grade and summing these values; thus, for each patient at a given sampling time, the composite score was between 0 and 400. Also the damage frequency was calculated; this represents the number of comets per 100 examined nucleoids.

The cellular nickel and chromium contents were measured by using graphite furnace-atomic absorption spectrometry (Varian, Mulgrave, Australia).

The amounts of cell suspension containing 1000 cells were calculated and treated with 100 μL of concentrated nitric acid. The samples were then left for 3 days at

room temperature for complete digestion.[49] The sample was diluted online 1 + 4 by the auto sampler with double-distilled water. A normal calibration curve was constructed for each element by 4 points including the calibration blank of the element standard (Merck, Whitehouse Station, NJ) for nickel and chromium to give 0, 2.5, 5, and 10 μg per liter of the standard solutions of the elements. The calibration curve plotted the absorption area signal of solutions of known element concentrations.

Statistical analysis

The statistical analysis was performed with the Statistical Package for the Social Sciences (version 17.01, SPSS, Chicago, Ill). Initially, the control and treatment groups were tested for homogeneity by using the Levene test for the equality of variance. Changes in the treatment and control groups across the time line were evaluated for each variable. The comparisons were done between values at T0 and T1, between T1 and T2, and between T0 and T2. This had the advantage of comparing the same subject with time to limit interindividual variations. Normally distributed variables (composite score and damage frequency) were tested with paired *t* tests. Viability %, and cellular nickel and chromium contents did not follow a Gaussian distribution and were assessed with the Wilcoxon signed rank test. When the control group showed changes with time, the relative risk was calculated by using the chi-square and Fisher exact tests to compare the incidence of occurrence of a suspected end point in the control and treatment groups. Changes in each test group were also evaluated across the time line with the Wilcoxon signed rank test. Simple linear regression was used to test the effect of cellular metal content (nickel and chromium) on the other variables (viability %, composite score, and damage frequency). The correlation coefficient was calculated for the different variables at the sampling periods (T0, T1, and T2). Measurement of error was calculated for cellular metal content by remeasuring nickel and chromium in 33 randomly selected samples, and the readings were compared with the Wilcoxon signed rank test. All tests were 2-tailed and were evaluated at the 95% CI of the differences.

RESULTS

In the treatment group (Table II), the viability % values were 8.1 ± 6.1 at T0 and decreased to 6.4 ± 3.8 at T1. However, a significant decrease was recorded only at T2 (4.5 ± 2.8). The composite score showed a significant decrease at T2 in the treatment group from 125.6 ± 46.05 to 98.8 ± 33.7. The cellular metal content (nickel and chromium) increased significantly. Nickel and chromium at T0 were 0.52 ± 0.32 and 0.31 ± 0.26 ng per milliliter, respectively. The values of nickel at T1 and T2 were 0.68 ± 0.28 and 0.78 ± 0.19 ng per milliliter, respectively, and chromium contents were 0.41 ± 0.28 ng per milliliter at T1 and 0.58 ± 0.26 ng per milliliter at T2.

Table II. Changes in the test group at T0, T1, and T2

Test group (n = 28)

Variable	*Mean (± SD)*	*Z*	P *value*
Viability %			
T1	6.44 (±3.83)	−1.755	0.079
T0	8.11 (±6.14)		
T2	4.51 (±2.77)	−2.620	0.009*
T0	8.11 (±6.14)		
Cellular nickel content (ng/mL)			
T1	0.6795 (±0.283)	−4.283	0.000†
T0	0.5196 (±0.316)		
T2	0.7810 (±0.191)	−3.212	0.001†
T1	0.6795 (±0.283)		
T2	0.7810 (±0.191)	−3.850	0.000†
T0	0.5196 (±0.316)		
Cellular chromium content (ng/mL)			
T1	0.4090 (±0.276)	−3.645	0.000†
T0	0.3063 (±0.255)		
T2	0.5839 (±0.257)	−3.121	0.002*
T1	0.4090 (±0.276)		
T2	0.5839 (±0.257)	−4.146	0.000†
T0	0.3063 (±0.255)		
	Mean (± SD)	t	P *value*
Composite score			
T1	108.4 (±54.06)	−1.351	0.188
T0	125.6 (±46.05)		
T2	98.8 (±33.70)	−2.814	0.009*
T0	125.6 (±46.05)		
Damage frequency (%)			
T1	45.71 (±17.80)	−0.290	0.774
T0	46.71 (±14.33)		
T2	42.57 (±10.34)	−1.251	0.222
T0	46.71 (±14.33)		

Viability %, Number of viable cells/total counted cells × 100; *damage frequency*, number of comets/100 nucleoids × 100; *ng/mL*, nanograms per milliliter.
*Significant at $P < 0.01$; †Significant at $P \leq 0.001$.

The control group (Table III) showed significant decreases in the composite score and damage frequency at T1. Composite score decreased from 108 ± 30.9 to 50.9 ± 27.1 at T1. The damage frequency decreased from 37.3 ± 11.1 at T0 to 27.4 ± 10.4 at T1. Changes in viability % and cellular metal content (nickel and chromium) were insignificant.

The relative risk showed significant differences between the control and treatment groups at T1 for the composite score (Fig), damage frequency, and cellular

Table III. Changes in the control group at T0, T1, and T2

	Control (n = 18)		
Variable	*Mean (±SD)*	*Z*	P *value*
Viability %			
T1	6.7 (±3.5)	−0.414	0.679
T0	6.2 (±3.5)		
T2	5.2 (±3.7)	−1.026	0.305
T0	6.2 (±3.5)		
Cellular nickel content (ng/mL)			
T1	0.58 (±0.28)	−1.112	0.266
T0	0.51 (±0.37)		
T2	0.53 (±0.35)	−1.417	0.157
T0	0.51 (±0.37)		
Cellular chromium content (ng/mL)			
T1	0.29 (±0.16)	−0.283	0.777
T0	0.31 (±0.17)		
T2	0.29 (±0.12)	−0.632	0.527
T0	0.31 (±0.17)		
	Mean (±SD)	t	P *value*
Composite score			
T1	50.9 (±27.1)	−6.104	0.000†
T0	108 (±30.9)		
T2	101.1 (±33.6)	−0.836	0.415
T0	108 (±30.9)		
Damage frequency (%)			
T1	27.4 (±10.4)	−3.527	0.003*
T0	37.3 (±11.1)		
T2	40.2 (±7.6)	0.777	0.448
T0	37.3 (±11.1)		

Viability %, Number of viable cells/total counted cells × 100; *damage frequency*, number of comets/100 nucleoids × 100; *ng/mL*, nanograms per milliliter.
*Significant at $P < 0.01$; †Significant at $P \leq 0.001$.

chromium content (Table IV). The relative risk at T2 showed no significant differences between the control and the treatment groups for any tested variables.

Each treatment group was compared with its values at T0 for all tested variables to evaluate any changes.

In group 1 (StSt-StSt), the only toxic changes were decreases in viability % at T1 and T2 (Table V). Significant increases in cellular nickel and chromium contents at both T1 and T2 were detected when compared with concentrations at T0 (Tables VI and VII). There was no correlation between the increases in nickel or chromium levels and the decrease in viability of the buccal mucosa cells.

In group 2 (StSt-NiTi), significant decreases in the composite scores at T1 and T2 compared with T0 (Table VIII) were seen. The cellular nickel content showed significant increases at T1 and T2 compared with T0 values (Table VI). Cellular chromium content also showed significant increases at T1 and T2 compared with T0 (Table VII). A strong inverse correlation was seen between cellular nickel content and damage frequency ($r = -1$, significant at $P < 0.01$) at T1. Also, inverse correlations were recorded at T2 between the cellular nickel content and the composite score ($r = -1$, significant at $P < 0.01$), and the cellular chromium content and the damage frequency ($r = -1$, significant at $P < 0.01$).

In group 3 (Ti-StSt), damage frequency showed significant increases at T1 and T2 (Table IX). Cellular nickel and chromium contents (Tables VI and VII) increased at both T1 and T2 but were statistically significant only at T1. Neither nickel nor chromium correlated to the damage frequency at T1. However, at T2, nickel was highly correlated to the increase in damage frequency ($r = 1$, significant at $P = 0.01$).

In group 4 (Ti-NiTi), significant decreases in viability % were detected at T1 and T2 (Table V). The composite score at T2 and the damage frequency at T1 and T2 both decreased (Tables VIII and IX). The increases in cellular nickel and chromium content (Tables VI and VII) were not significant at either T1 or T2.

The error of measurement calculated for the cellular metal content showed insignificant differences for the 2 sets of readings at $P < 0.05$ (Table X).

DISCUSSION

To evaluate the cytotoxicity and genotoxicity of fixed orthodontic appliances on buccal mucosa cells, a prospective longitudinal clinical investigation was carried out with a treated group and a control group with similar criteria over a 6-month period. We also further investigated whether combinations of various materials of brackets and archwires produced different effects. Any toxic change was correlated to the cellular nickel and chromium content of the buccal mucosa cells. To our knowledge, this is the only longitudinal in-vivo study evaluating orthodontic patients with different bracket and archwire combinations for a duration of 6 months.

In-vitro studies testing material biocompatibility lack the simulation of the oral cavity with its multifactorial environment. It is difficult to produce a similar corrosive environment and to select a suitable cell type. Testing the toxicity is difficult, since variable cell types show different sensitivities and tolerances.[50]

On the other hand, in-vivo studies are not without flaws because biologic variations introduced by each patient affect the standardization of the study. This, however, seemed advantageous because it allowed the evaluation of the effects of fixed orthodontic appliances in their natural functional environments. Most studies evaluated the corrosion by-products in the saliva. This might have limitations, since the saliva is continuously washed and swallowed, and will give information at

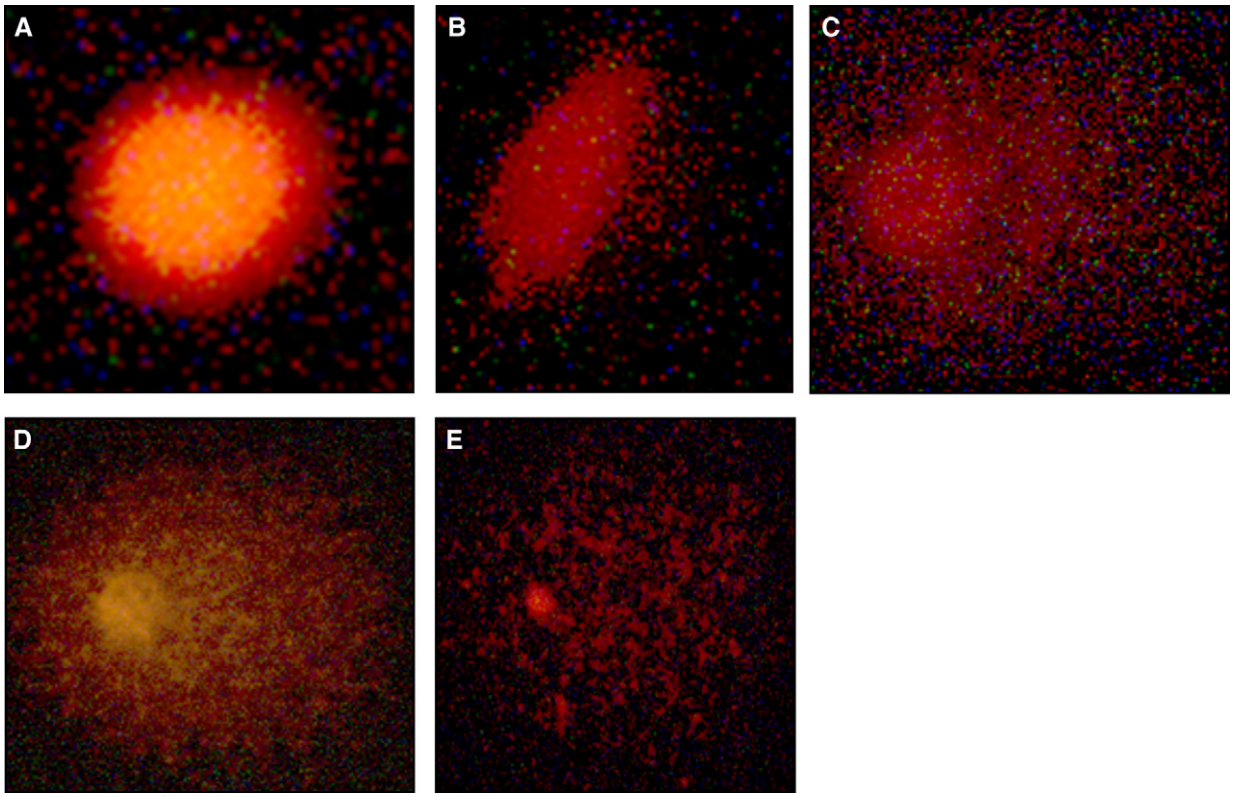

Fig. Nucleoids showing varying amounts of DNA damage: **A,** grade 0, no damage; **B,** grade 1, DNA fragmentation up to 10%; **C,** grade 2, fragmentation of 10% to 50%; **D,** grade 3, fragmentation of 50% to 75%; and **E,** grade 4, fragmentation above 75%.

Table IV. Significant differences between relative risks in control and treatment group after 3 months

Variable	*Treatment period*	*Relative risk*	*CI*		P *value*
Composite score	T1	0.56	0.38	0.84	0.017*
Damage frequency (%)	T1	0.54	0.30	0.97	0.04*
Cellular chromium content (ng/mL)	T1	0.32	0.11	0.91	0.038*

Damage frequency, Number of comets/100 nucleoids × 100; *ng/mL*, nanograms per milliliter.
*Significant at $P < 0.05$.

the moment of sampling only.[11] Thus, the use of buccal mucosa cells seemed advantageous because they are in direct contact with the appliance, and it has been reported that oral tissues uptake the metal ions released by adjacent metal restorations.[19] Therefore, the oral tissues might show the cumulative effects of metal release.

Although in-vivo studies are extremely beneficial in explaining how oral tissues and orthodontic materials react in their actual functioning environment, the interpretation of the results is usually difficult because of many factors not under experimental control. Individual variability was seen for most of the variables evaluated in this study. To eliminate interindividual variations, the patients were evaluated longitudinally to act as their own controls, so that these variables were negligible in the overall assessment. Because of the great interindividual variations and large standard deviations, many variables were insignificant when comparing the means of the treatment group with those of the control group. However, significant differences were noted when comparing these variables with their average values at T0. Because of the longitudinal nature and the restrictive criteria for sample selection in this study, the sample size was small. This compounded the drawback of large standard deviations, and the results should be evaluated with caution. The control group without orthodontic

Table V. Viability % (cellular viability) changes with different bracket-archwire combinations at T0, T1, and T2

Group	Variable	Mean (±SD)	Z	P value
Group 1 StSt-StSt (n = 8)	T1	7.8 (±6.4)	−2.111	0.035*
	T0	10.2 (±8.0)		
	T2	4.1 (±2.9)	−2.111	0.035*
	T0	10.2 (±8.0)		
Group 2 StSt-NiTi (n = 6)	T1	5.2 (±2.6)	−0.106	0.916
	T0	6.9 (±8.5)		
	T2	4.5 (±2.3)	−0.106	0.916
	T0	6.9 (±8.5)		
Group 3 Ti-StSt (n = 6)	T1	6.1 (±2.2)	−0.740	0.459
	T0	5.5 (±4.2)		
	T2	5.7 (±3.0)	−0.106	0.916
	T0	5.5 (±4.2)		
Group 4 Ti-NiTi (n = 8)	T1	6.3 (±2.1)	−2.132	0.033*
	T0	9.0 (±2.3)		
	T2	4.1 (±3.1)	−2.533	0.011*
	T0	9.0 (±2.3)		

Viability %, Number of viable cells/total counted cells × 100.
*Significant at $P < 0.05$.

Table VI. Cellular nickel content changes with different bracket-archwire combinations at T0, T1, and T2

Group	Variable	Mean (±SD) (ng/mL)	Z	P value
Group 1 StSt-StSt (n = 8)	T1	0.63 (±0.24)	−2.533	0.011*
	T0	0.40 (±0.26)		
	T2	0.76 (±0.27)	−2.111	0.035*
	T1	0.63 (±0.24)		
	T2	0.76 (±0.27)	−2.533	0.011*
	T0	0.40 (±0.26)		
Group 2 StSt-NiTi (n = 6)	T1	0.57 (±0.17)	−2.220	0.026*
	T0	0.43 (±0.18)		
	T2	0.64 (±0.07)	−2.220	0.026*
	T1	0.57 (±0.17)		
	T2	0.64 (±0.07)	−2.220	0.026*
	T0	0.43 (±0.18)		
Group 3 Ti-StSt (n = 6)	T1	0.91 (±0.44)	−2.220	0.026*
	T0	0.69 (±0.51)		
	T2	0.88 (±0.13)	−0.740	0.459
	T0	0.69 (±0.51)		
Group 4 Ti-NiTi (n = 8)	T1	0.64 (±0.18)	−1.548	0.122
	T0	0.58 (±0.23)		
	T2	0.84 (±0.15)	−1.548	0.122
	T0	0.58 (±0.23)		

ng/mL, Nanograms per milliliter.
*Significant at $P < 0.05$.

treatment was included to determine that changes were unique for the treatment group and that the control group did not react in a similar manner.

In some orthodontic patients, allergy has been a documented reaction. Although a problem, the true concern should be the possible cytotoxicity or, even more importantly, the genotoxicity of orthodontic appliances. Persistent DNA damage can lead to mutations. In a labile tissue such as the buccal mucosa, cellular proliferation of a damaged cell might cause many defective cells.[29] Cellular toxicity will also affect the cells' metabolism and, in turn, their function and repair capacity.

Some general trends were recorded for the treatment group. Cytotoxicity was denoted by a significant decrease in cellular viability. Cellular viability at T0 in the treatment group was low. When compared with the T0 value (Table II), the viability decreased significantly at T2. Low cellular viability of the normal buccal mucosa cells was a common finding reported by other authors.[44,51,52] However, other studies showed much greater cellular viability.[18,53] Faccioni et al[18] reported similar cytotoxicity for the treated group in their study, when the cellular viability decreased significantly. Other in-vivo studies also reported decreases in cellular viability and metabolism in orthodontic patients.[36,37] Cellular alterations included changes in metabolism, alterations in the regularity of cells, increases in the cellular nuclear-cytoplasmic area ratio, and larger cells with pyknotic and vacuolated nuclei. Some studies[30,54] showed increases of cellular proliferation with low nickel concentrations, but this was only initially, and then the cellular viability decreased.[31,33]

The composite score (Table II) in the treatment group at T2 showed a significant decrease compared with the T0 value. This indicates the possible genotoxic effect of orthodontic appliances. As pointed out, DNA damage can take the form of DNA fragmentation and increases in migration and comet formation, producing a higher composite score. On the other hand, metal ions can cause DNA damage by decreasing DNA migration through crosslinking DNA fragments either by DNA-DNA or DNA-protein crosslinking. A decrease in the composite score without a decrease in damage frequency (the number of nucleoids showing comets in 100 cells) as seen in this study might indicate crosslinking of the DNA.

DNA damage was also suggested by Faccioni et al,[18] who saw an increase in the number of comets. However, their study showed fewer comets for both the control and the treated groups, at 11.43 ± 6.58 and 17.62 ± 10.08, respectively. Westphalen et al[38] also recorded

Table VII. Cellular chromium content changes with different bracket-archwire combinations at T0, T1, and T2

Group	Variable	Mean (±SD) (ng/mL)	Z	P value
Group 1 StSt-StSt (n = 8)	T1	0.44 (±0.13)	−2.533	0.011*
	T0	0.29 (±0.08)		
	T2	0.70 (±0.25)	−2.533	0.011*
	T1	0.44 (±0.13)		
	T2	0.70 (±0.25)	−2.533	0.011*
	T0	0.29 (±0.08)		
Group 2 StSt-NiTi (n = 6)	T1	0.29 (±0.12)	−2.220	0.026*
	T0	0.20 (±0.16)		
	T2	0.57 (±0.14)	−2.220	0.026*
	T1	0.29 (±0.12)		
	T2	0.57 (±0.14)	−2.220	0.026*
	T0	0.20 (±0.16)		
Group 3 Ti-StSt (n = 6)	T1	0.6 (±0.53)	−2.220	0.026*
	T0	0.46 (±0.50)		
	T2	0.57 (±0.35)	−1.586	0.113
	T0	0.46 (±0.50)		
Group 4 Ti-NiTi (n = 8)	T1	0.33 (±0.12)	−0.422	0.673
	T0	0.29 (±0.11)		
	T2	0.49 (±0.25)	−1.126	0.260
	T0	0.29 (±0.11)		

ng/mL, Nanograms per milliliter.
*Significant at $P < 0.05$.

DNA damage in their orthodontic patients at 30 days of treatment with the micronucleus test. However, the comet assay showed insignificant changes. The numbers of comets were low before and 10 days after treatment at 1.5 ± 1.03 and 2.5 ± 3.08, respectively. However, they evaluated only 50 nucleoids, not 100 per sample, and for a treatment duration of only 10 days.

The measured cellular nickel and chromium contents (Table II) showed significant increases at T1 and T2 when compared with the T0 values. There were also significant increases in both nickel and chromium at T2 when compared with T1, denoting a steady increase over time. Unlike other studies that showed only significant initial increases of metals and then decreases or stability over time, in our investigation, there were continuous metal increases up to 6 months.[3,7] The T0 values were much lower than the amounts of metals found in the other studies.

Significant changes were seen in the control group for composite score and damage frequency at T1 (Table III). To compare these changes with those in the treatment group, the relative risk was calculated; this compares the frequency of incidence of changes in each group. The relative risk indicated

Table VIII. Composite score changes with different bracket-archwire combinations at T0, T1, and T2

Group	Variable	Mean (±SD)	t	P value
Group 1 StSt-StSt (n = 8)	T1	108.5 (±73.1)	−0.262	0.801
	T0	116.3 (±43.6)		
	T2	80.5 (±21.0)	−2.144	0.069
	T0	116.3 (±43.6)		
Group 2 StSt-NiTi (n = 6)	T1	68 (±18.1)	−3.559	0.016*
	T0	124 (±33.4)		
	T2	92 (± 8.0)	−2.688	0.043*
	T0	124 (±33.4)		
Group 3 Ti-StSt (n = 6)	T1	124.7 (±13.9)	0.423	0.690
	T0	117.3 (±36.9)		
	T2	141.3 (±42.0)	1.221	0.277
	T0	117.3 (±36.9)		
Group 4 Ti-NiTi (n = 8)	T1	126.5 (±59.6)	−0.568	0.587
	T0	142.3 (±63.5)		
	T2	90.3 (±24.0)	−3.106	0.017*
	T0	142.3 (±63.5)		

*Significant at $P < 0.05$.

Table IX. Damage frequency (%) changes with different bracket-archwire combinations at T0, T1, and T2

Group	Variable	Mean (±SD)	t	P value
Group 1 StSt-StSt (n = 8)	T1	48.3 (±25.3)	0.877	0.410
	T0	40.5 (±10.0)		
	T2	38.5 (±9.0)	−0.991	0.355
	T0	40.5 (±10.0)		
Group 2 StSt-NiTi (n = 6)	T1	39.3 (±12.5)	−1.645	0.161
	T0	49.3 (±20.5)		
	T2	42.3 (±4.0)	−1.035	0.348
	T0	49.3 (±20.5)		
Group 3 Ti-StSt (n = 6)	T1	48.0 (±2.7)	10.157	0.000‡
	T0	38.7 (±2.3)		
	T2	56.3 (±6.8)	4.806	0.005†
	T0	38.7 (±2.3)		
Group 4 Ti-NiTi (n = 8)	T1	46.3 (±20.5)	−2.557	0.038*
	T0	57.0 (±12.7)		
	T2	36.5 (±7.9)	−4.425	0.003†
	T0	57.0 (±12.7)		

Damage frequency, Number of comets/100 nucleoids ×100.
*Significant at $P < 0.05$; †Significant at $P < 0.01$; ‡Significant at $P < 0.001$.

a significant difference between the changes in the patients compared with the control group for the composite score, the damage frequency, and cellular chromium content at T1 (Table IV). However, the relative risk showed no significant differences between

Table X. Measurement of errors for nickel and chromium concentrations

	First readings	*Second readings*		
Variable	*Mean (±SD)*	*Mean (±SD)*	*Z*	P *value*
Cellular nickel content (ng/mL)	0.796 (±0.58)	0.733 (±0.47)	−1.646	0.100*
Cellular chromium content (ng/mL)	0.369 (±0.29)	0.359 (±0.26)	−482	0.629*

*Nonsignificant at $P < 0.05$.

Table XI. Summary of changes in the treatment groups with different bracket-archwire combinations

Variables	*Group 1 StSt-StSt*	*Group 2 StSt-NiTi*	*Group 3 Ti-StSt*	*Group 4 Ti-NiTi*
Viability % (cellular viability)	3 months: ↓	3 months: NS	3 months: NS	3 months: ↓
	6 months: ↓	6 months: NS	6 months: NS	6 months: ↓
Composite score	3 months: NS	3 months: ↓	3 months: NS	3 months: NS
	6 months: NS	6 months: ↓	6 months: NS	6 months: ↓
Damage frequency	3 months: NS	3 months: NS	3 months: ↑↑↑	3 months: ↓
	6 months: NS	6 months: NS	6 months: ↑↑	6 months: ↓↓
Cellular nickel content	3 months: ↑	3 months: ↑	3 months: ↑	3 months: NS
	6 months: ↑	6 months: ↑	6 months: NS	6 months: NS
Cellular chromium content	3 months: ↑	3 months: ↑	3 months: ↑	3 months: NS
	6 months: ↑	6 months: ↑	6 months: NS	6 months: NS

↓Decrease significant at $P < 0.05$; ↓↓Decrease significant at $P \leq 0.01$; ↑Increase significant at $P \leq 0.05$; ↑↑Increase significant at $P \leq 0.01$; ↑↑↑Increase significant at $P < .001$; *NS*, Nonsignificant at $P \leq 0.05$.

the control and the treated groups for any variables at T2. This might indicate that, after the initial damage produced by the metal ions, cellular adaptation or repair occurred.

Specific changes were seen for the different bracket-archwire combinations. Group 1 (StSt-StSt) had significant decreases in cellular viability at T1 and T2 (Table V). This was similar to the decreased viability and cellular metabolism reported by David and Lobner[55] for stainless steel archwires; they implicated nickel and chromium released from stainless steel archwires for the decreases in cellular viability and metabolism. No DNA damage was reported for group 1. In group 2 (StSt-NiTi), a genotoxic effect was denoted by the significant decrease in the composite score at T1 and T2 compared with T0 (Table VIII), but no significant change in cellular viability was recorded (Table V). In groups 1 and 2, cellular nickel and chromium contents significantly increased at T1 and T2 in comparison with their T0 values (Tables VI and VII).

In group 3 (Ti-StSt), significant increases in the damage frequency at T1 and T2 (Table IX) indicated DNA damage. Nickel and chromium (Tables VI and VII) showed significant increases only at T1 when compared with the T0 measurements. Compared with group 1, in which a stainless steel bracket was used with a similar archwire, the cellular metal content was significant for a shorter duration when titanium brackets were used.

In group 4 (Ti-NiTi), a decrease in cellular viability was recorded (Table V). DNA damage was also indicated by significant decreases in the composite score and the damage frequency (Tables VIII and IX). The increases in cellular nickel and chromium content were not significant at either T1 or T2 (Tables VI and VII). The combination of titanium brackets and nickel titanium archwires had the highest toxicity despite the insignificant releases of nickel and chromium. The amounts of ions released for this group were similar to the amounts measured for the other groups reporting significant differences; however, the large standard deviations in group 4 might have prevented the detection of significant differences (Tables VI and VII). Other studies have reported subtoxic effects at low metal concentrations.[31-33,35,36,38,56] The cytotoxic changes found in this study were contrary to the findings by Gil et al,[57] who showed no cellular toxic changes by either titanium or nickel-titanium materials in vitro.

Titanium alloys are known for their biocompatibility.[58] However, in this study, the proven biocompatibility did not guarantee no biologic changes when titanium brackets were combined with different archwires. This suggests that material biocompatibility must be evaluated in combinations similar to those used during orthodontic treatment.

Although significant correlations were recorded between cellular metal content and biologic changes, it

does not indicate a cause-and-effect relationship. As verified by the linear regression analysis, in this sample, neither nickel nor chromium was the cause of any of the cytotoxic or genotoxic changes. This might be explained by the relatively small sample size combined with large standard deviations. Significant relationships might be seen in a larger sample.

This study showed significant changes occurring in orthodontic patients treated with fixed appliances over a 6-month period. Different combinations of brackets and archwires produced variable effects (Table XI). These changes included increases in cellular nickel and chromium content, decreases in cellular viability, and evidence of DNA damage. The fate of these changes needs follow-up, since repair of biologic changes is possible. When DNA damage occurs, various reparative mechanisms are regularly activated to maintain the integrity of the DNA.[21,25,27] However, the persistence of DNA damage will lead to genetic instability and DNA mutations.[29,59] It is documented that metal ions interfere with many protective and reparative pathways that maintain cellular homeostasis and DNA integrity.

Based on the recorded biologic changes, it would be prudent to reduce any insult induced by the orthodontic appliances. This can be achieved by the adoption of treatment techniques that reduce the duration of treatment and the enforcement of higher standards for corrosion resistance of orthodontic appliance by the manufacturers.

CONCLUSIONS

1. In this study, buccal mucosa cells of patients treated with fixed orthodontic appliances for 6 months showed significant increases in nickel and chromium content, with significant decreases in viability and damage to the DNA.
2. When these results were compared with those from the control group, the changes were significant only for cellular chromium content and DNA damage at 3 months. This might imply recovery from the initial insult by cellular and DNA tolerance or repair.
3. Stainless steel brackets with stainless steel archwires showed the least biologic damage, whereas the titanium brackets with nickel-titanium archwires produced the greatest cytotoxicity and genotoxicity.

REFERENCES

1. Anusavice K. Phillips' science of dental materials. 11th ed. Philadelphia: W. B. Saunders; 2003: p. 57-64.
2. Eliades T, Bourauel C. Intraoral aging of orthodontic materials: the picture we miss and its clinical relevance. Am J Orthod Dentofacial Orthop 2005;127:403-12.
3. Ağaoğlu G, Arun T, İzgü B, Yarat A. Nickel and chromium levels in the saliva and serum of patients with fixed orthodontics appliances. Angle Orthod 2001;71:375-9.
4. Fors R, Persson M. Nickel in dental plaque and saliva in patients with and without orthodontic appliances. Eur J Orthod 2006;28:292-7.
5. Menezes L, Quintão C, Bolognese A. Urinary excretion levels of nickel in orthodontic patients. Am J Orthod Dentofacial Orthop 2007;131:635-8.
6. Petoumenou E, Arndt M, Keilig L, Reimann S, Hoederath H, Eliades T, et al. Nickel concentration in the saliva of patients with nickel-titanium orthodontic appliances. Am J Orthod Dentofacial Orthop 2009;135:59-65.
7. Singh D, Sehgal V, Pradhan K, Chandna A, Gupta R. Estimation of nickel and chromium in saliva of patients with fixed orthodontic appliances. World J Orthod 2008;9:196-202.
8. Souza R, Menezes L. Nickel, chromium and iron levels in the saliva of patients with simulated fixed orthodontic appliances. Angle Orthod 2008;78:345-50.
9. Arndt M, Brück A, Scully T, Jäger A, Bourauel C. Nickel ion release from orthodontic NiTi wires under simulation of realistic in-situ conditions. J Mater Sci 2005;40:3659-67.
10. Barret R, Bishara S, Quinn J. Biodegradation of orthodontic appliances. Part I. Biodegradation of nickel and chromium in vitro. Changes in the blood level of nickel. Am J Orthod Dentofacial Orthop 1993;103:8-14.
11. Eliades T, Trapalis C, Eliades G, Katsavarias E. Salivary metal levels of orthodontic patients: a novel methodological and analytical approach. Eur J Orthod 2003;25:103-6.
12. Es-Souni M, Es-Souni M, Fischer-Brandies H. Assessing the biocompatibility of NiTi shape memory alloys used for medical applications. Anal Bioanal Chem 2005;381:557-67.
13. Huang TH, Yen CC, Kao CT. Comparison of ion release from new and recycled orthodontic brackets. Am J Orthod Dentofacial Orthop 2001;120:68-75.
14. Huang TH, Ding SJ, Min Y, Kao CT. Metal ion release from new and recycled stainless steel brackets. Eur J Orthod 2004;26:171-7.
15. Hwang CJ, Shin JS, Cha JY. Metal release from simulated fixed orthodontic appliances. Am J Orthod Dentofacial Orthop 2001;120:383-91.
16. Sfondrini M, Cacciafesta V, Maffia E, Massironi S, Scribante A, Alberti G, et al. Chromium release from new stainless steel, recycled and nickel-free orthodontic brackets. Angle Orthod 2009;79:361-7.
17. Amini F, Farahani A, Jafari A, Rabbani M. In vivo study of metal content of oral mucosa cells in patients with and without fixed orthodontic appliances. Orthod Craniofac Res 2008;11:51-6.
18. Faccioni F, Franceschetti P, Cerpelloni M, Fracasso M. In vivo study on metal release from fixed orthodontic appliances and DNA damage in oral mucosa cells. Am J Orthod Dentofacial Orthop 2003;124:687-94.
19. Garhammer P, Schmalz G, Hiller KA, Reitinger T. Metal content of biopsies adjacent to dental cast alloys. Clin Oral Invest 2003;7:92-7.
20. Wataha J. Biocompatibility of dental casting alloys: a review. J Prosthet Dent 2000;83:223-34.
21. Rojas E, Herrera L, Poirier L, Ostrosky-Wegman P. Are metals dietary carcinogens? Mutat Res 1999;443:157-81.
22. Schmalz G, Garhammer P. Biological interactions of dental cast alloys with oral tissues. Dent Mater 2002;18:396-406.
23. Setcos J, Babaei-Mahanib A, Di Silvio L, Mjörd I, Wilson N. The safety of nickel containing dental alloys. Dent Mater 2006;22:1163-8.
24. House K, Sernetz F, Dymock D, Sandy J, Ireland A. Corrosion of orthodontic appliances—should we care? Am J Orthod Dentofacial Orthop 2008;133:584-92.

25. Beyersmann D, Hartwig A. Carcinogenic metal compounds: recent insight into molecular and cellular mechanism. Arch Toxicol 2008; 82:493-512.
26. Desoize B. Cancer and metals and metal compounds: part I—carcinogenesis. Crit Rev Oncol Hematol 2002;42:1-3.
27. Seiler H, Sigel A, Sigel H. Handbook on metals in clinical and analytical chemistry. New York: Marcel Dekker; 1994.
28. International Agency for Research on Cancer. IARC Monographs on the evaluation of carcinogenic risks to humans. Lyon; 1990.
29. Mitchell R, Cotran R. Cell injury, adaptation and death. In: Kumar V, Cotran R, Robbins S, editors. Robbins basic pathology. 7th ed. Philadelphia: W. B. Saunders; 2003. p. 4-11.
30. El Medawar L, Rocher P, Hornez JC, Traisnel M, Breme J, Hildebrand H. Electrochemical and cytocompatibility assessment of NITINOL memory shape alloy for orthodontic use. Biomol Eng 2002;19:153-60.
31. Taira M, Toguchi M, Hamada Y, Okazaki M, Takahashi J, Ito R, et al. Studies on cytotoxicity of nickel ions using C3H10T1/2 fibroblast cells. J Oral Rehab 2000;27:1068-72.
32. Trombetta D, Mondello M, Cimino F, Cristani M, Pergolizzi S, Saija A. Toxic effect of nickel in an in vitro model of human oral epithelium. Toxicol Lett 2005;159:219-25.
33. Wataha J, Lockwood P, Schedle A, Noda M, Bouillaguet S. Ag, Cu, Hg, and Ni ions alter the metabolism of human monocytes during extended low-dose exposures. J Oral Rehab 2002;29:133-9.
34. Pizzoferrato A, Cenni E, Ciapetti G, Granchi D, Savarino L, Stea S. Inflammatory response to metals and ceramics. In: Barbucci R, editor. Integrated biomaterials science. New York: Kluwer Academic/Plenum Publishers; 2002. p. 735-91.
35. Lü X, Bao X, Huang Y, Qu Y, Lu H, Lu Z. Mechanisms of cytotoxicity of nickel ions based on gene expression profiles. Biomaterials 2009;30:141-8.
36. Kasacka I, Szarmach IJ, Buczko P, Tankiewicz A, Pawlak D. Preliminary evaluation of morphological parameters of the saliva in patients undergoing orthodontic treatment. Adv Med Sci 2006; 51(Suppl 1):52-4.
37. Pereira B, Tanaka O, Lima A, Guariza-Filho O, Maruo H, Camargo E. Metal and ceramic bracket effects on human buccal mucosa epithelial cells. Angle Orthod 2009;79:373-9.
38. Westphalen G, Menezes L, Prá D, Garcia G, Schmitt V, Henriques J, et al. In vivo determination of genotoxicity induced by metals from orthodontic appliances using micronucleus and comet assays. Genet Molec Res 2008;7:1259-66.
39. Reichart P, Philipsen H. Color atlas of dental medicine. New York: Thieme; 2000: p. 63.
40. Ribeiro D, Salvadori D, de Assis G, Marques M. Does fluoride cause DNA damage? An in vitro evaluation using rats oral mucosa cells. Braz J Oral Sci 2003;2:268-71.
41. Ribeiro D, Bazo AP, da Silva Franchi C, Marques M, Salvadori D. Chlorhexidine induces DNA damage in rat peripheral leukocytes and oral mucosal cells. J Periodontal Res 2004;39:358-61.
42. Dusinska M, Collins A. The comet assay in human biomonitoring: gene-environment interactions. Mutagenesis 2008;23:191-205.
43. Nia A, Van Straaten H, Godschalk R, Van Zandwijk N, Balm A, Kleinjans J, et al. Immunoperoxidase detection of polycyclic aromatic hydrocarbon-DNA adducts in the mouth floor and buccal mucosa cells of smokers and nonsmokers. Environ Molec Mutagen 2000;36:127-33.
44. Proia N, Paszkiewicz G, Nasca M, Franke G, Pauly J. Smoking and smokeless tobacco-associated human buccal cell mutations and their association with oral cancer—a review. Cancer Epidemiol Biomarkers Prev 2006;15:1061-77.
45. Albertini R, Anderson D, Douglas G, Hagmar L, Hemminki K, Merlo F, et al. IPCS guidelines for the monitoring of genotoxic effects of carcinogens in humans. Mutat Res 2000;463:111-72.
46. Strober W. Common immunologic techniques: trypan blue exclusion test of cell viability. In: Coligan J, Kruisbeek A, Margulies D, Shevach E, Strober W, editors. Current protocols in immunology. Vol. 3. New York: John Wiley; 1996; A.3B 1-2.
47. Tice R, Vasquez M. Protocol for the application of the pH< 13 alkaline single cell gel (SCG) assay to the detection of DNA damage in mammalian cells. Durham, NC: Integrated Laboratory Systems, 1998.
48. Szeto Y, Benzie I, Collins A, Choi S, Chenga C, Yowa C, et al. A buccal cell model comet assay: development and evaluation for human biomonitoring and nutritional studies. Mutat Res 2005; 578:371-81.
49. Garhammar P, Hiller KA, Reitinger T, Schmaltz G. Metal content of saliva of patients with and without metal restorations. Clin Oral Invest 2004;8:238-42.
50. Issa Y, Brunton P, Waters C, Watts D. Cytotoxicity of metal ions to human oligodendroglial cells and human gingival fibroblasts assessed by mitochondrial dehydrogenase activity. Dent Mater 2008;24:281-7.
51. Oßwald K, Mittas A, Glei M, Pool-Zobel B. New revival of an old biomarker: characterisation of buccal cells and determination of genetic damage in the isolated fraction of viable leucocytes. Mutat Res 2003;544:321-9.
52. Yamamoto M, Saeki K, Utsumi K. Isolation of human salivary polymorphonuclear leukocytes and their stimulation-coupled responses. Arch Biochem Biophys 1991;289:76-82.
53. Rojas E, Valverde M, Sordo M, Ostrosky-Wegman P. DNA damage in exfoliated buccal cells of smokers assessed by the single cell gel electrophoresis assay. Mutat Res 1996;370:115-20.
54. Gursoy U, Sokucu O, Uitto VJ, Aydin A, Demirer S, Toker H, et al. The role of nickel accumulation and epithelial cell proliferation in orthodontic treatment-induced gingival overgrowth. Eur J Orthod 2007;529:555-8.
55. David A, Lobner D. In vitro cytotoxicity of orthodontic archwires in cortical cell cultures. Eur J Orthod 2004;26:421-6.
56. Costa M, Lenza M, Gosch C, Costa I, Ribeiro-Dias F. In vitro evaluation of corrosion and cytotoxicity of orthodontic brackets. J Dent Res 2007;86:441-5.
57. Gil F, Solano E, Peña J, Engel E, Mendoza A, Planell J. Microstructural, mechanical and citotoxicity evaluation of different NiTi and NiTiCu shape memory alloys. J Mater Sci Mater Med 2004;15:1181-5.
58. Elias C, Lima J, Valiev R, Meyers M. Biomedical applications of titanium and its alloys. JOM 2008;60:46-9.
59. Ribeiro D, Salvadori D, da Silva R, Darros B, Marques M. Genomic instability in non-neoplastic oral mucosa cells can predict risk during 4-nitroquinoline 1-oxide-induced rat tongue carcinogenesis. Oral Oncol 2004;40:910-5.