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Original Research Article

Atomic density of elements on the surface of orthodontic bands

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ABSTRACT

The study was performed on new and used (from *in vitro* and *in vivo* experiments) orthodontic bands by using SEM-EDX technique. The bands were retrieved from earlier experiments: *in vitro* tests, in which the bands were incubated in a continuous flow system with various media: artificial saliva, lysozyme, orange juice and Coca Cola®. The bands were also retrieved from previously conducted *in vivo* tests on animals (pigs) and humans (patients). The micrographs of bands were presented as well as their chemical composition, reported in terms of atomic density. The bands that were used showed a significant contribution of oxygen as compared to brand new ones, and the contribution of Fe and Ni decreased, whereas the Cr contribution remained unchanged. The elements were inter-correlated. An antagonistic, statistically significant dependence was found between Fe and O, as well as between Fe and Cr. This could signify that that protective passivation layer of Cr_2O_3 was formed, which did not fully protect Ni and Fe from dissolution.

1. Introduction

Metallic devices (brackets, wires and bands) which constitute the orthodontic fixed appliances are held in the oral cavity for 2 years on average treatment. Corrosion is one of the major concerns related to biocompatibility of metallic biomaterials, because impacts the release of ions [1,2]. Chemical composition has very much to do with corrosion. Orthodontic stainless steel alloys usually contain Fe, Cr, Ni, Co, Si which can be transferred to the human organism during corrosion [3–5]. Some of the elements mentioned above are known as cytototoxic, mutagenic and sensitizing agents [6]. High degree of biocompatibility is being expected from orthodontic materials because of prolonged contact with the surrounding tissues.

The existing ISO standards are not obligatory for manufactures and the companies can create their own standards. Resulting, it is reported that some manufacturers do not pay sufficient attention to the final processing stages (finishing). The consequence could be the lower biocompatibility [7].

The evaluation of the characteristics of orthodontic materials is an important step in understanding the mechanisms of metal ions release under *in vitro* and *in vivo* conditions. Various properties play an important role in the search for ideal materials (*e.g.* biostability). Surface properties (roughness, surface topography, elemental composition) affect the biocompatibility and the performance, as well as

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corrosion and esthetics. Moreover, surface roughness influences plaque accumulation [8]. Orthodontic alloys are exposed to various substances in oral cavity: saliva which becomes acidic in contact with food and activity of microflora in biofilm (biodeterioration) [6].

Metallic orthodontic devices with a high surface area exposed to the oral environment can cause a problem because of the possibility of adverse biological effects due to the release of heavy metal ions (corrosion and wear) [9].

The biocompatibility of the material is related to the passive film present on the surface. Chromium forms a thin and adherent oxide Cr_2O_3-based passive layer that provides corrosion resistance by blocking the diffusion of oxygen into the basic bulk alloy [3,6]. A minimum of 12 percent of chromium is bound to transmitting the necessary corrosion resistance [10]. This factor is essential for the preservation of biological materials. Changes in the passive layer make material susceptible to corrosion [11].

The aim of the present study was evaluation of atomic density of elements on the surface of orthodontic bands by the SEM-EDX technique exposed to various conditions of *in vitro* and *in vivo* (patients, animals) experiments.

2. Materials and methods

2.1. *Tested material*

The evaluated materials were new and used orthodontic bands (size 37+; 3M Unitek, Monrovia, CA, USA). The latter were obtained from the orthodontic appliances of three series of experiments (*in vitro* tests with various solutions, animal tests and *in vivo* tests on humans). Wires, brackets and bands were all made of stainless steel. The chemical composition (%) of bands, provided by the manufacturer was: 65 Fe, 17 Cr, 12 Ni, 2.5 Mo, 2 Mn, 1 Si, 0.045 P, 0.03 C, 0.03 S.

2.2. In vitro *tests*

Four series of *in vitro* tests were performed (with (I) artificial saliva, (II) artificial saliva with lysozyme, (III) orange juice and artificial saliva, (IV) Coca Cola® and artificial saliva). The orthodontic appliance which consisted of two wires, four bands, 20 brackets and 20 elastic ligatures, were placed in the thermostatic glass reactor assuring the conditions of continuous flow of artificial saliva, and incubated at 37 °C for 28 days. In the first and second series, artificial saliva and artificial saliva with lysozyme, respectively, was flowing through the system with the flow rate reflecting the flow of saliva in the human oral cavity (0.5 mL/min). The details of the experiment and *in vitro* system were described in previous studies [12]. In the third and fourth series, orange juice and Coca Cola® (330 mL), respectively, were flowing through the system for 5.5 h (1.0 mL/min) every day, while artificial saliva – for the rest of day (0.5 mL/min) [13].

2.3. In vivo *test on animals*

The animal experiment was conducted on pigs, chosen as a model organism. The plates that aimed to simulate the orthodontic appliance were made of bands and were placed on the buccal side of pig's cheek for 6 months. The details of the experiment were described earlier [14].

2.4. In vivo *test on humans*

Used bands were collected from orthodontic patients after 12 months of treatment. The details of the experiment were described earlier [15].

2.5. *Analytical methods*

The external surface of the new and used orthodontic bands was evaluated by the SEM-EDX technique. Before SEM-EDX analysis, all samples were degreased with ethanol. Samples were mounted on an appropriate stub and were subjected to Roentgen microanalysis using the Phenom ProX desktop scanning electron microscope with BSD detector, operating at EHT = 15 kV. For each orthodontic band five analyses were performed.

2.6. *Statistical methods*

The results were elaborated statistically by *Statistica ver. 10.0*. Descriptive statistics (means, standard deviations) were reported. The normality of distribution of the experimental results was assessed by the Shapiro–Wilk test. Statistical differences between new and used bands were assessed by the Tukey test and Kruskal–Wallis test. Results were considered significantly different when $p < 0.05$.

3. Results and discussion

Evaluated bands were retrieved from the experiments conducted earlier: *in vitro* (artificial saliva, artificial saliva with orange juice or Coca Cola®) and *in vivo* (patients, animals – pigs). Previously, metal ions release in these studies was discussed. The total mass of released metal ions during 4 weeks of the *in vitro* test in the continuous flow system (in the environment of artificial saliva) was: nickel 18.7 μg, chromium 5.47 μg and copper 31.3 μg [12]. Similar experiments were conducted with the use of soft drinks (orange juice and Coca Cola®, respectively). The total mass of ions released was, μg: Ni (15.33; 37.75), Cr (3.604; 1.052), Fe (48.42; ≥156.1), Cu (57.87, 32.91), Mn (9.164; 41.16), Mo (9.999; 30.12), Cd (0.5967; 2.173). It was found that orange juice did not intensify the release of metal ions from orthodontic appliances, whereas Coca Cola® caused increased release of Ni ions [13]. *In vivo* experiments (conducted on animals) revealed that Ni and Cr were released and accumulated in various tissues. The sites of accumulation were: aorta (4.8 times higher of Ni), in the cheek (Ni 3.5 times higher), and in the hair sampled after 3 months (Cr 3.4 times higher), as related to the control group. The doses of toxic metal ions released from the appliance did not reach toxic levels [14]. The trials on patients were conducted. Hair was sampled in time (1 year period) as a non-invasive biomarker of exposure. The following masses of ions were transferred to hair tissue: 7.42 ± 14.19 μg of Ni, 8.94 ± 13.1 μg of Cr, and 131 ± 279 μg of Fe. The content of Cr was statistically significantly

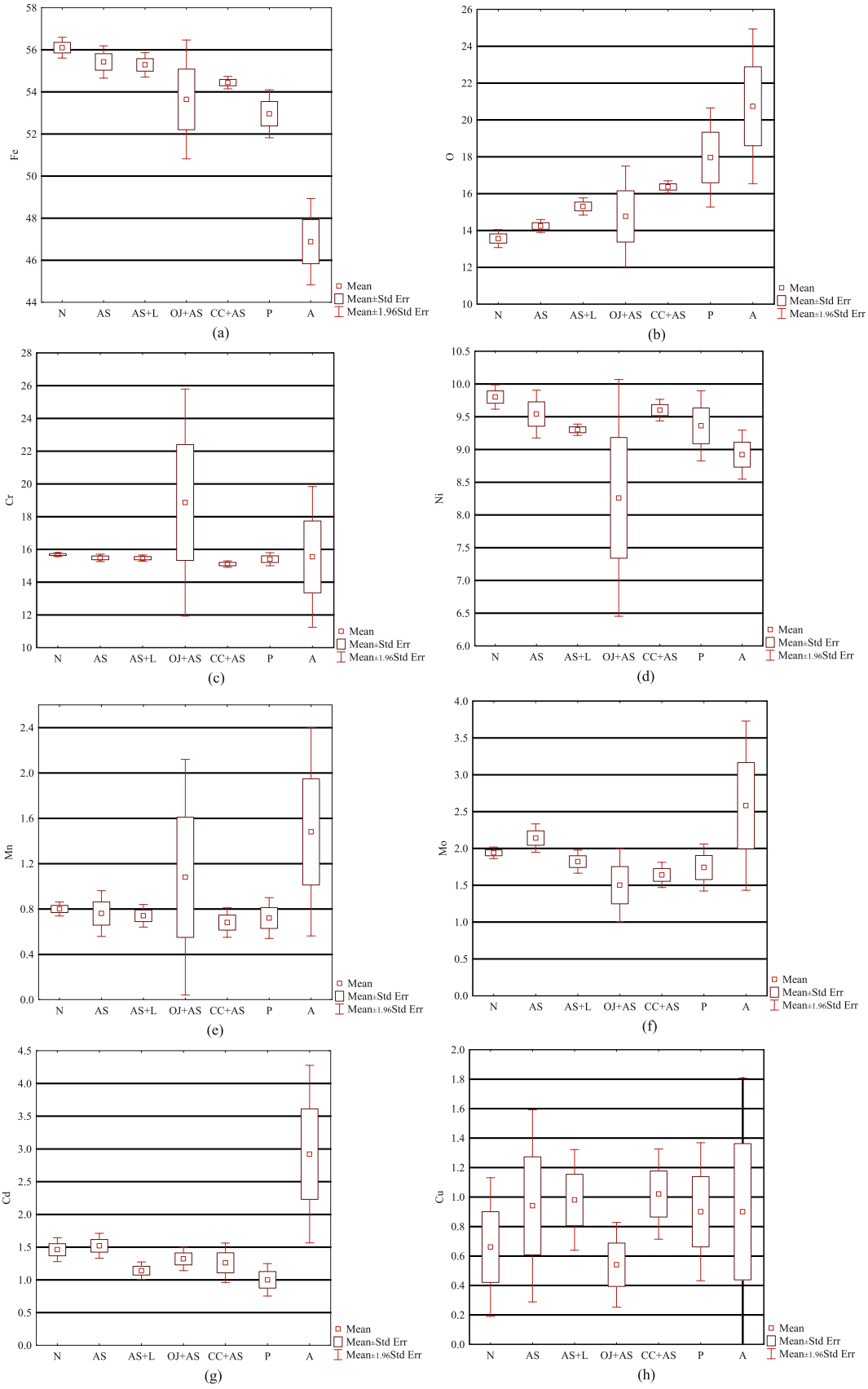

Fig. 1 – Atomic density of elements (%) on the surface of orthodontic bands; N – new, AS – artificial saliva, AS + L – lysozyme in artificial saliva, OJ + AS – orange juice and artificial saliva, CC + AS – Coca Cola® and artificial saliva, P – patient, A – animal (pig).

higher during treatment than before the beginning of therapy [15].

To combine the results of all the above experiments, a mathematical kinetic model that linked the results of *in vitro* and both *in vivo* trials was developed [16].

The present work reports the comparison of elemental surface composition (by SEM-EDX) of the bands used in the experiments described above. By using the SEM, the microstructure of stainless steel alloy specimens (atoms) can be visualized.

Table 1 reports results of elemental analyses by SEM-EDX: mean results, standard deviations are reported, as well as statistical analysis of the results. Box-plot graphs of the elemental composition of bands are shown in Fig. 1. The highest differences were found between the bands that were present in pigs' snout and the new ones. Fig. 2 presents SEM photographs of bands (new, from patients, from animals (pig)). No distinction could be made between irregularities on the surface caused by handling during the manufacturing and caused by orthodontic treatment. Surface alterations originated from corrosion, wear and deformation. The formation of biofilm is responsible for an increased contribution of C, O, Ca and P [17]. Fig. 3 shows correlations between atomic density of elements: metals (Ni, Cr, Fe) and oxygen. High and statistically significant correlations (antagonistic) were found between Fe and O ($r = 0.900$) and between Cr and Ni ($r = 0.835$). This means that along with the increased contribution of O (from 13 up to 21%), the contribution of Fe decreased (from 56 down to 47%). The level of Cr did not change. The contribution of Ni slightly decreased with the increase in O (from 9.8 to 8.9%). This could justify the formation of the protective layer of Cr_2O_3 together with the dissolution of Ni and Fe ions from stainless steel. Previously [18] a decrease in chromium and iron contribution to the surface, with the increase in oxygen content in used *vs.* new elements of the appliance was found. Our results confirm the formation of oxides (passivation layer) on the surface of stainless steel as a result of the presence of the orthodontic appliance in patients' oral cavities.

In the discussion on the elemental density of surface materials, it is necessary to take into account the fact that the

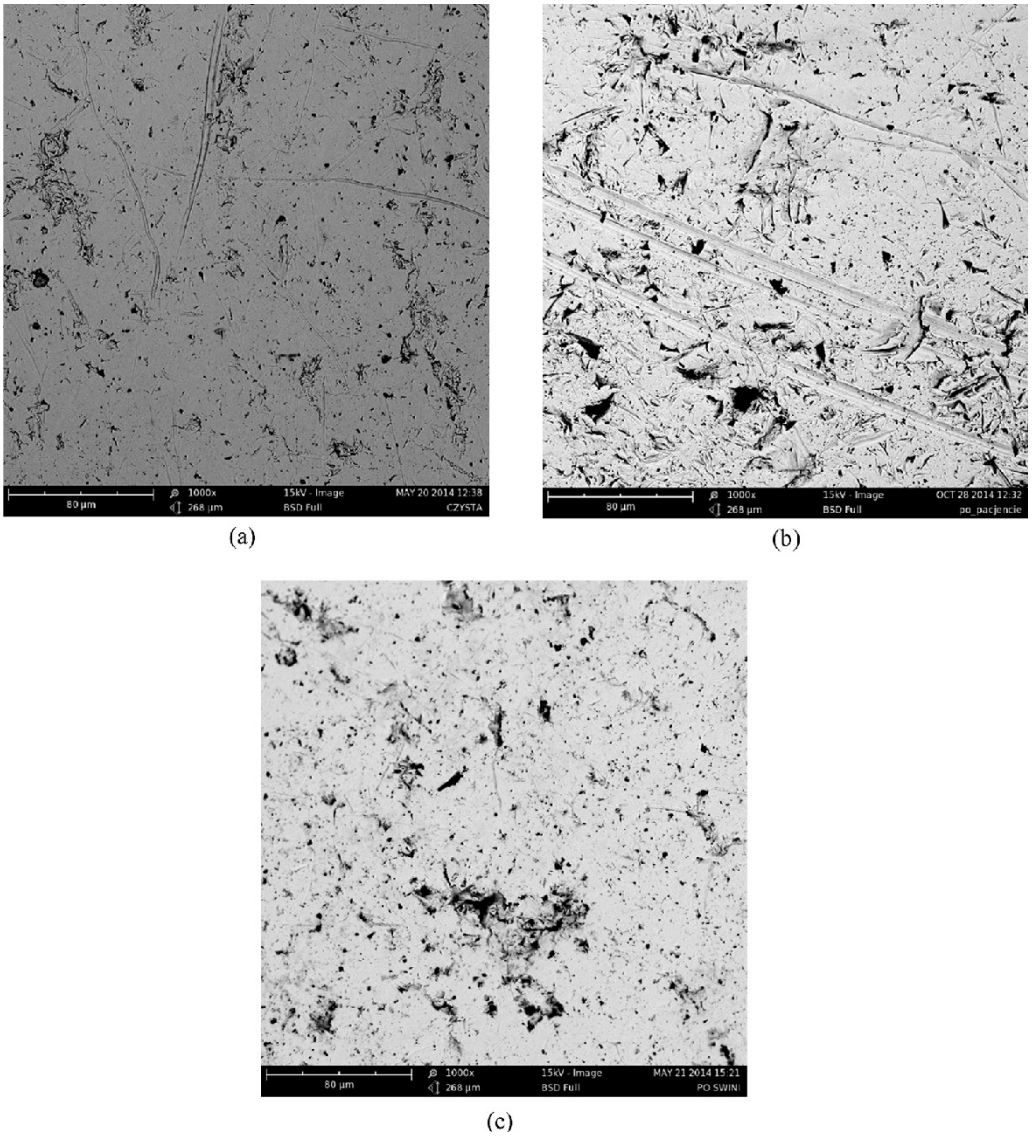

Fig. 2 – SEM photographs of bands (a) new, (b) patient, and (c) animal (pig).

Table 1 – Atomic density of elements (%) on the surface of bands.

	New		Artificial saliva		Artificial saliva with lyzosyme		Orange juice and artificial saliva		Coca Cola and artificial saliva		Patient		Animal (pig)	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Fe*	$55.1^{A,a}$	0.6	55.4^{B}	0.9	55.3^{C}	0.7	53.6^{D}	3.2	54.4^{E}	0.3	$53.0^{F,a}$	1.3	$46.9^{A,B,C,D,E,F}$	2.3
O**	$13.6^{A,B,a}$	0.6	14.2	0.4	15.3	0.5	14.8	3.1	16.4^{a}	0.4	18.0^{A}	3.1	20.7^{B}	4.8
Cr**	15.7	0.1	15.5	0.3	15.5	0.2	18.9	7.8	15.1	0.2	15.4	0.4	15.5	4.9
Ni**	$9.80^{A,a}$	0.21	9.54	0.42	9.30	0.10	8.26^{a}	2.06	9.60	0.19	9.36	0.61	8.92^{A}	0.43
Mn**	0.800	0.071	0.760	0.230	0.740	0.114	1.08	1.19	0.680	0.145	0.720	0.205	1.48	1.05
Mo**	1.94	0.09	2.14	0.22	1.82	0.18	1.50	0.57	1.64	0.19	1.74	0.36	2.58	1.31
Cd**	1.46	0.21	1.52	0.22	1.14	0.15	1.32	0.20	1.26	0.34	1.00^{a}	0.28	2.92^{a}	1.54
Cu*	0.660	0.537	0.940	0.744	0.980	0.390	0.540	0.329	1.02	0.35	0.900	0.534	0.900	1.034

$n = 5$. *Tukey test. **Kruskal–Wallis test, $^{A,B,C}p < 0.05$, $^{a,b}p < 0.1$.

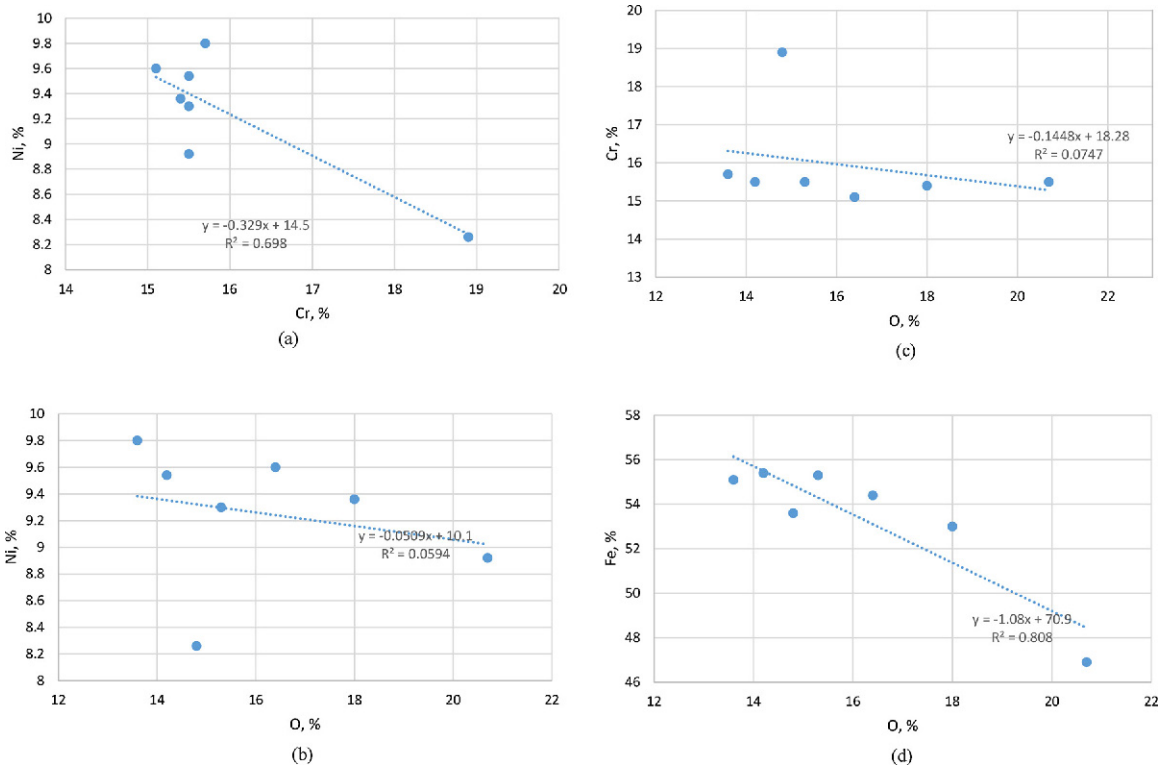

Fig. 3 – Correlations between atomic density of elements on the surface of bands.

results are not reported as concentrations, but as atomic surface density. This means that the increase in the contribution of one element (*e.g.* oxygen) may result in a decrease in the atomic density of another, but it does not necessarily mean that the element was released, but actually covered by another element. This makes the interpretation of the results difficult.

In the case of iron, statistically significant differences were found between bands in pigs and patients on the one hand and new bands on the other. The elemental density of iron in bands from pigs was by 14.9% lower than in the new ones. Differences between bands from patients and new bands were denoted (3.8% lower). This confirms the release of iron from orthodontic appliances as the result of corrosion that occurred to a higher extent in pigs, probably because of a higher contribution of biofilm microflora.

The contribution of oxygen reflects the passivation of the surface of bands. Statistically higher levels were in bands from pigs, patients and the *in vitro* experiments with Coca Cola®. The higher contribution of oxygen was found in bands from pig's snout (a 52.2% higher level than that in new bands). In the bands from patients the contribution of oxygen was by 32.3% higher than in new ones. In the bands form the *in vitro* Coca Cola® study, the contribution of oxygen increased by 20.6%.

Surprisingly, the contribution of Cr on the surface did not change statistically significantly. It increased in bands from the orange juice from *in vitro* trial (a 20.4% increase) as compared with the new bands. In the case of nickel, the

statistically significant differences were found between bands from pigs (a 8.98% decrease) and orange juice (15.7%).

For Mn, Mo, Cd and Cu there were no statistically significant differences between new bands and the remaining groups. The only statistical difference was found for Cd in pigs (by 100% higher than in new) and patients (by 31.5% higher than in new). The contribution of Cd in pigs was by 192% higher than in patients. The origin of Cd seems to be unknown. The following differences were noticeable, but not statistically significant: Mg pig-new 85% higher, orange juice *vs.* new 35% higher. For Mo, the contribution in bands from pigs was 33% higher than in new. The contribution of Cu was higher in bands from pigs (36.4%) and in patients (36.4%) as compared with the new bands.

In the study conducted by Mendes et al. [19], evaluating chemical composition of orthodontic brackets (ABNT 316 steel) after 12–24 months of use, the following elements were detected: Fe (70.2%), Cr (19.5%), Mo (0.8%), and Ni (8.8%). The authors stated that the accumulation of deposits masked the original topography of the brackets' surfaces. Some elements appeared on the surface of brackets and it was impossible to determine their origin: Cu, Ag and Al [19].

The activity of microorganisms lowers pH that initiates corrosion through deterioration of the protective passivation layer [6]. Additionally, the passive layer consisting of Cr_2O_3 is destructed by mechanical and chemical factors (fluorides from toothpastes and mouthwashes, as well as carbonate drinks) [13,20]. As a result, metal ions are being dissoluted: Fe, Ni and Cr which, depending on the dose, could cause sensitizing, carcinogenic or mutagenic effects [6].

4. Conclusions

Orthodontic bands retrieved from previous *in vitro* and *in vivo* (animals, patients) experiments were evaluated by SEM-EDX technique that enabled investigation of changes in atomic density of elements on their surface. It was found that SEM-EDX analysis is a valuable tool for the characterization of the distribution of elements and quantitative analysis for studies on corrosion. Changes in the elemental composition of the surface reflected the formation of passivation film, adhering the surface of materials: the contribution of oxygen increased and iron and nickel decreased, while Cr was unchanged. These changes, as well as analysis of inter-element correlations, suggested that the film consisting of chromium oxide is formed, which protects from corrosion by blocking the transport of oxygen to the alloy which determines its biocompatibility.

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