MORPHOLOGICAL ASPECT AND SURFACE CHEMICAL ANALYSIS OF SALIVARY CRYSTALS IN YOUNG PATIENTS WITH/WITHOUT ORTHODONTIC APPLIANCES

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Abstract

Our study analyzes the morphological aspect and surface elemental chemical composition of salivary crystals obtained from 19 subjects (11 males and 8 females) aged 11-26 years (mean age 20.64±5.316 years) with/without orthodontic appliances, by the SEM-EDS method. Statistical evaluation was performed using the SPSS 20.0 software. Three morphological types of salivary crystals were identified, namely: type I, with regulated polyhedral aspect, formed of a complex chemical compound with a high percentage of potassium and chlorine; type II, with irregular ovoid aspect, formed of a complex chemical compound with a high percentage of sodium, oxygen and silicon; type III, with ovoid luminescent layout, formed of a complex chemical compound with a high percentage of phosphorus, oxygen and potassium. Statistically significant differences were found between the mean values of nickel and calcium on the groups of subjects without and with previously applied orthodontic appliances ($p<0.05$).

Keywords: *salivary crystals, morphological aspect, surface chemical analysis, orthodontic appliances, SEM-EDS method.*

1. INTRODUCTION

Human saliva is a clear, slightly acidic mucoserous exocrine secretion, consisting of 99% water and 1% dry residue composed of inorganic (chlorides, bicarbonates, phosphates of sodium, potassium and calcium) and organic substances (enzymes, proteins and immunoglobulins) [1,2]. The salivary function has to maintain oral health and to create an appropriate ecological balance [2,3]. Collection of unstimulated saliva is the simplest non-invasive method for its research.

Orthodontic appliances are temporarily applied in the oral cavity of patients, in order to correct malocclusions and dental malpositions. Removable appliances consist of an acrylic baseplate and metallic elements of stainless steel (SS) alloy (screw, clasps and springs). Metallic brackets, bands, arches, springs and ligature wires of fixed appliances are made of SS alloy, and some archwires of Nickel-Titanium (NiTi) alloy [4,5].

Previous studies have shown that, in saliva, the SS and NiTi alloys become corrosive [6-8], reducing their biocompatibility and releasing metal ions into it [9-13]. Other authors investigated the allergic, carcinogenic, mutagenic and cytotoxic effects of nickel, but the results obtained were controversial [14-17].

The objective of our study is to analyze crystallized unstimulated human saliva of subjects with/without orthodontic appliances and to identify the morphological aspect and surface elemental chemical composition of salivary crystals, by the SEM-EDS method.

2. MATERIALS AND METHODS

Saliva was collected from 19 subjects (11 males and 8 females), with ages between 11-26 years (mean age 20.64±5.316 years) from northeastern Romania. The subjects were divided into three groups, as follows: 5 (26.32%) subjects without orthodontic appliances, 8 (73.68%) with orthodontic appliances and 6 (31.58%) with previously applied orthodontic appliances. The selection criteria were: no health problems, no medication and no other substances administered intra-orally and without piercings. The informed consent was obtained before salivary collection from all subjects.

Unstimulated saliva was collected from each patient, on a 0.5 mm glass slide, previously degreased and decontaminated. Collecting was done in the morning, between 10-11 am, after teeth brushing. The salivary samples were dried in a controlled environment, under the dome, in order to avoid atmospheric deposits.

The morphological characteristics of crystals of salivary samples were observed with Scanning Electron Microscopy (SEM), on a Quanta 200 3D microscope (FEI Company, Holland). 4-6 salivary crystals taken over from different regions of each salivary sample were examined and 110 SEM photomicrographs were obtained for the groups of subjects. The surface elemental chemical composition of crystals of salivary sampling was evaluated by EDS analysis (Energy Dispersive X-ray Spectrum), the mass (Wt%) and atomic (At%) percentage being determined. To avoid measurement errors caused by the presence of organic material in the analyzed saliva, measurements were carried out without taking into account the chemical element carbon.

Statistical analysis was performed in SPSS 20.0 (SPSS Inc., Chicago, IL) for Windows. Data was characterized by descriptive statistics, frequency distributions and contingency tables. To compare the numerical variables between the groups of subjects, we used the *t*-student test for independent samples, assuming that the normal distribution law is verified (as confirmed by the Kolmogorov-Smirnov fitting test). To estimate the confidence intervals (CI) 95% of the mean values, standard deviations (SD) and standard errors (SE) were used by the bootstrapping procedure, for 1000 bootstrap samples. The entire statistical analysis was performed with a 5% level of significance and the *p*-value <0.05 was regarded as significant.

3. RESULTS

The photomicrographs showed salivary crystals with various shapes and sizes, including rectangular, polyhedral, ovoid, rhomboid and rod-like forms, separated or congested in the organic matter of dried saliva. According to their morphological characteristics, three main salivary crystals types were considered for analysis:

a) type I, big size crystals with polyhedral regulated (rectangular, polyhedral) shape, single or in cluster arrangements (92.72% salivary crystals) (Figs. 1-2);
b) type II, big and medium size crystals with irregular shape (irregular polyhedron, ovoid, rod-like forms), in cluster arrangements (2.72% salivary crystals) (Fig. 3);
c) type III, isolated, medium and small size crystals with irregular shape (irregular polyhedron, ovoid, rod-like forms), characterized by luminescence phenomena when analyzed by scanning electron microscopy (4.54% salivary crystals) (Fig. 4).

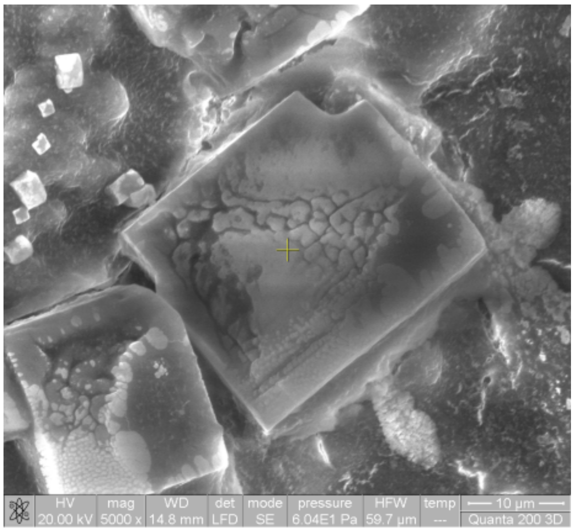

Fig. 1. SEM photomicrography of rectangular single salivary crystals type I (Original magnification x5000)

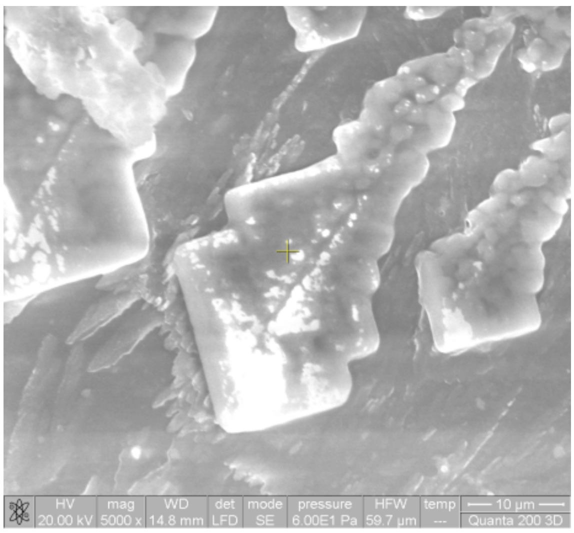

Fig. 2. SEM photomicrography of type I salivary crystals, polyhedral in cluster arrangements (Original magnification x5000)

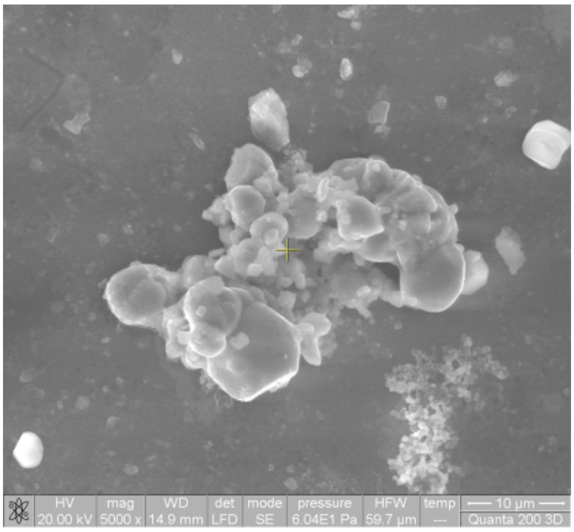

Fig. 3. SEM photomicrography of type II ovoid salivary crystals in cluster arrangements (Original magnification x5000)

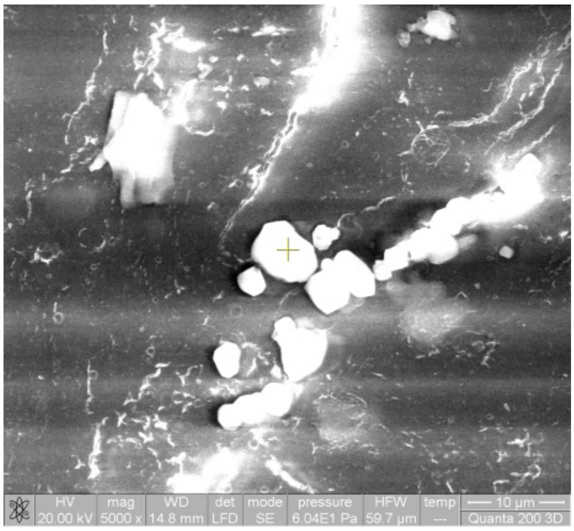

Fig. 4. SEM photomicrography of type III luminescence ovoid salivary crystals (Original magnification x5000)

The surface elemental chemical composition of the three identified morphological types of salivary crystals was analyzed (Table 1). Increased average values were recorded for:

a) chlorine (36.07 Wt% and 32.52 At%) and potassium (35.36 Wt% and 29.08 At%) of the salivary crystals type I;
b) oxygen (19.25 Wt% and 31.12 At%), sodium (19.19 Wt% and 23.42 At%) and silicon (22.86 Wt% and 24.23 At%) of the salivary crystals type II;
c) oxygen (30.42 Wt% and 47.80 At%), phosphorus (19.22 Wt% and 15.54 At%) and potassium (32.20 Wt% and 20.88 At%) of the salivary crystals type III.

Table 1. Surface chemical composition of salivary crystals types

Surface chemical elemental composition		**Salivary crystals** (n=110)					
		Type I (n=102)		**Type II** (n=3)		**Type III** (n=5)	
		Mean	SD	Mean	SD	Mean	SD
O	Wt%	10.0057	3.37535	19.2467	7.83881	30.4180	5.04272
	At%	19.5565	5.47284	31.1233	10.32730	47.8000	6.41941
Ni	Wt%	3.5237	0.89927	1.9367	1.09701	1.2860	0.72518
	At%	1.9055	0.49856	0.9167	0.60995	0.5540	0.30063
Na	Wt%	2.6714	0.85837	19.1867	13.23705	3.1280	1.81512
	At%	3.6463	1.01145	23.4267	18.88265	3.2800	2.19695
Mg	Wt%	1.0204	0.20134	2.5400	0.25000	0.9760	0.39361
	At%	1.3282	0.24094	2.8033	0.58020	1.0080	0.45185
Al	Wt%	1.0567	0.25849	0.9500	0.07937	0.9020	0.44082
	At%	1.2458	0.29783	1.7533	1.47886	0.8460	0.41356
Si	Wt%	7.1859	4.40783	22.8600	12.33635	6.6240	1.84309
	At%	7.9473	4.53622	24.2333	15.05842	6.0020	1.88124

P	Wt%	1.3757	0.75439	1.9833	1.77652	19.2180	5.35134
	At%	1.4246	0.74910	1.6600	1.39861	15.5420	3.97207
S	Wt%	1.1135	0.60515	0.6967	0.60435	1.8500	2.56746
	At%	1.0978	0.54381	0.5400	0.46861	1.5200	2.18573
Cl	Wt%	36.0786	6.55971	5.4667	4.41608	3.1560	0.50471
	At%	32.5225	6.48965	3.8633	3.07419	2.2520	0.40221
K	Wt%	35.3631	7.12598	5.3333	3.77170	32.2040	3.88738
	At%	29.0825	6.68491	3.4467	2.32707	20.8840	3.37095
Ca	Wt%	0.4831	0.97591	7.7333	7.89466	0.4320	0.96598
	At%	0.3816	0.77354	4.8233	4.94413	0.2920	0.65293
Zn	Wt%	0.0079	0.06613	12.0700	20.90585	0.00	0.00
	At%	0.0134	0.10975	5.5700	9.64752	0.00	0.00

The surface elemental chemical composition of salivary crystals on the groups of subjects was analyzed (Table 2), statistically significant differences being observed between the mean values of nickel and calcium on the groups of subjects without orthodontic appliances and with previously applied orthodontic appliances ($p<0.05$) (Table 3). Comparison between the mean values of chemical composition of salivary crystals showed no statistically significant differences ($p>0.05$).

Table 2. Surface chemical composition of salivary crystals on groups of subjects

Surface chemical elemental composition		**Salivary crystals on groups of subjects** (n=110)					
		without appliances		**with appliances** (n=43)		**with previously applied appliances** (n=36)	
		(n=31)	SD	Mean	SD	Mean	SD
O	Wt%	11.0035	3.38666	11.4835	6.07716	10.9864	6.88720
	At%	21.1410	5.38211	21.6551	8.48540	20.5719	10.13582
Ni	Wt%	3.0429	0.99068	3.3863	0.93353	3.6589	1.12247
	At%	1.6355	0.57502	1.8356	0.55430	1.9514	0.60725
Na	Wt%	4.2597	5.67671	2.7726	2.09729	2.6225	0.88023
	At%	5.6890	7.40685	3.6388	2.19416	3.4936	0.95556
Mg	Wt%	1.0794	0.36906	1.0516	0.37848	1.0528	0.20607
	At%	1.3748	0.43629	.43629	0.38904	1.3558	0.25232
Al	Wt%	0.9826	0.22604	1.0440	0.21370	1.1053	0.33719
	At%	1.2210	0.50340	1.2177	0.26831	1.2875	0.37215
Si	Wt%	8.7984	5.66232	7.1588	5.81780	7.0578	3.97941
	At%	9.5406	5.83554	7.8837	6.39304	7.7381	3.93504
P	Wt%	1.3258	1.59224	2.3093	4.44509	2.8322	4.63320
	At%	1.4077	1.50859	2.1181	2.1181	2.5911	3.64277
S	Wt%	1.0168	1.07276	1.1058	0.49424	1.2736	0.78044
	At%	0.9848	0.91448	1.0849	0.49009	1.2228	0.65685

Cl	Wt%	31.6477	9.15556	33.0963	9.42176	36.3328	12.39857
	At%	28.1313	8.80989	29.9709	9.12004	32.7589	11.48573
K	Wt%	34.0552	7.09998	35.7251	7.04862	33.1158	10.86731
	At%	27.5268	6.19571	28.9714	6.69830	27.2797	10.21043
Ca	Wt%	1.2684	1.27687	0.8214	2.67677	0.00	0.00
	At%	0.9923	1.01409	0.5602	1.69896	0.00	0.00
Zn	Wt%	1.1942	6.49975	0.00	0.00	0.00	0.00
	At%	0.5832	2.99952	0.00	0.00	0.00	0.00

Table 3. The *t*-student test for comparing the mean values of nickel and calcium of salivary crystals on groups of subjects with/without orthodontic appliances

Chemical elements		*t*-student	*p* value	Mean difference	SE	95% CI	
						Lower	Upper
Ni	Wt%	-2.363	0.021*	-0.61599	0.26062	-1.13649	-0.09548
	At%	-2.176	0.033*	-0.31591	0.14520	-0.60589	-0.02592
Ca	Wt%	5.531	0.000**	1.26839	0.22933	0.80003	1.73675
	At%	5.448	0.000**	0.99226	0.18214	0.62029	1.36423

*statistically significant differences ($p<0.05$);
**extreme statistically significant differences ($p<0.0001$).

4. DISCUSSION

The morphological aspect and surface elemental chemical composition of salivary crystals have been investigated by the SEM-EDS method, to appreciate the differences observed in the ionic composition of normal human saliva, for bringing up new data to the existing literature.

Our study identified three morphological types of human salivary crystals, related to the surface elemental chemical composition. Type I of salivary crystal with regulated polyhedral aspect was formed of a complex chemical compound with a high percentage of potassium and chlorine. Type II of salivary crystals with irregular ovoid was formed of a complex chemical compound with a high percentage of sodium, oxygen and silicon. Type III of salivary crystals with ovoid luminescent layout was formed of a complex chemical compound with a high percentage of phosphorus, oxygen and potassium. These aspects have not been presented in the literature.

Previous studies have shown that the cation potassium and the ion phosphorus are predominant in human saliva, compared to the sodium cation. Sodium and chlorine ion concentration increases with the salivary flow rate, while the cation potassium is independent on the salivary flow [18,19].

Unlike other studies, the present investigation carried out surface elemental chemical analysis of salivary crystals for all chemical elements of the salivary components. Comparison of the mean values of salivary crystals' surface chemical elements on groups of subjects showed no statistical differences, with the exception of nickel and calcium.

Sodium, magnesium, phosphorus, sulfur, chlorine and potassium present in the salivary crystals derive from the ion content of human saliva. Calcium comes from the ionic content of human saliva, its concentration depending on age, pH and salivary flow [3,19]. Silicon may also come from dust particles in the atmosphere or from the glass composition of the samples used. Nickel, aluminum and zinc do not derive from the ionic content of saliva. Nickel was found in all groups of subjects studied, regardless of the presence/absence of orthodontic appliances in the oral cavity. A statistically significant difference was found for the groups of subjects without present orthodontic appliances, which indicates that they were not the source of nickel ions.

Previous studies have investigated the release of metal ions in saliva of patients with orthodontic appliances at different time intervals, compared to a control group without orthodontic appliances [20-25], but the results obtained were controversial. Most studies have reported an increased release of metal ions in saliva, caused by orthodontic appliances [26-29], due to the phenomenon of saliva corrosion [30,31], fluoride mouthwash and toothpaste [32,33] or potentiated by the effect of radiofrequency electromagnetic fields from mobile phones [34]. A group of authors have found a certain level of nickel in subjects with orthodontic appliances caused by the ingested diet composition [35].

Our research should convince orthodontic patients that orthodontic appliances have no influence upon their health status. Similar future studies may find other ways to investigate these patients, to obtain data comparable with ours.

5. CONCLUSIONS

The present research identified three morphological types with common types of salivary crystals formed of different percentages of complex chemical compounds. Some of the surface chemical elements of the salivary crystals derived from the ionic composition of human saliva. Ni was not released from orthodontic appliances in the oral cavity. The source of the metallic elements Ni, Al and Zn from the surface of the salivary crystals must be investigated by further research of the environment.

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