Digest Journal of Nanomaterials and Biostructures Vol. 9, No. 4, October – December 2014, p. 1429 - 1438

COMPLEX CHARACTERIZATION OF DENTAL OFFICE AEROSOLS REVEALS IMPORTANT LOADS OF RISK ELEMENTS FOR THE HUMAN HEALTH

M. R. SIMU[a*], C. BORZAN[b], M. MESAROS[a], M. T. CHIRIAC[c,d], T. RADU[e]
[a]*Department of Conservative Dentistry, Faculty of Dentistry, Iuliu Hatieganu University of Medicine and Pharmacy, 8 Babes Street, Cluj-Napoca, Romania*
[b]*Department of Community Medicine, Faculty of Medicine, Iuliu Hatieganu University of Medicine and Pharmacy, 8 Babes Street, Cluj-Napoca, Romania*
[c]*Department of Biology, Babes-Bolyai University, Cluj-Napoca, Romania*
[d]*Department of Medicine 1, University of Erlangen-Nuremberg, Erlangen, Germany*
[e]*Faculty of Physics & Interdisciplinary Research Institute on Bio-Nano-Sciences, Babes-Bolyai University, 1 M. Kogalniceanu, Cluj-Napoca, Romania*

Workplace conditions are directly reflected on the health of dental practitioners which in turn influences the quality of the medical services. Using a complementary approach our study aimed to determine the elemental composition, morphology and size distribution of non-microbial particles present in the air of a dental office, during various dental treatments, in order to identify and analyse potential risk factors for the human health. The samples were collected on carbon double adhesive tape - an original and very efficient particle collecting method. The aerosols produced during various treatments were analysed using the X–ray photoelectron spectroscopy (XPS), scanning electronic microscopy (SEM) and energy dispersive X-ray analysis (EDX). To the best of our knowledge, the present study is the first one to use the combination of these methods in studying dental aerosols. Using the highly sophisticated equipment allowed to augment the accuracy of our findings and helped to identify various types of elements, some of which were not reported in previous studies dealing with a similar problematic. Our results underline the aerosols potential of deeply penetrating into the respiratory system, even to the level of pulmonary alveoli, and thereby they represent serious health threats for the practitioners and patients.

(Received September 9, 2014; Accepted October 29, 2014)

Keywords: Dental aerosols; Biomaterials; Nanoparticles; Surface technique; Electronic microscopy

1. Introduction

The aerosols are defined as particles smaller than 50μm, i.e. small and light enough to rest in the breathable air for up to 30 minutes after their formation [1]. Hence, aerosols are considered to have the largest pathogenic potential, due to both their capacity to enter into the respiratory tract as a consequence of their small dimensions [2] and to their relatively long persistence in breathable air.

The composition of aerosols formed during different treatments in the dental office can be very complex, consisting of saliva particles, nasopharyngeal secretions, dental plaque, blood, dental substance, powders used at air abrasion method [1], metals from the composition of the dental turbine head or the low speed handpiece [3], materials from dental burs (tungsten) [4],

* Corresponding author: medaromana@yahoo.com

dental prosthetics, ceramic or metallic brackets during their removal, and different types of composites, glass ionomer cements, zinc oxide cement etc.

In our study we intended to determine to which extent the powders released after removal of fillings, preparing of the dental tissue for the prosthetic, endodontic or restorative treatment get close to the airways and to which level of the respiratory tree can reach depending on their dimension. Also we want to investigate the morphology and the elemental composition of the released particles during dental procedures.

The studies made up to now led to the same conclusion regarding the capacity of different instruments to contaminate the air with potential pathogen aerosols: firstly is the ultrasound scaling device, followed by the water cooling turbine, the air-abrasion device, the air-water spray. All of these produce a dense aerosol in the form of a visible cloud [5]. The most hazardous are the alveolar particles because they have dimensions smaller than 10μm (referred as PM10 ratio due to their aerodynamic diameter) which allow them to reach inside the lungs. Among these, the particles with diameter less than 2.5μm are the most critical because they can be deposited deeply in the lung alveoli [3]. Ballester et al. [6] claim that the gradual reduction of annual values of PM2.5 results in the diminishing of the number of premature deaths. During dental treatments the quantity of very small particles (< 0.5μm) which are released is much larger than that of particles > 0.5μm [7]. Concerning the hazard of aerosols exposure in the dental cabinet Taira [8] states that particles below 0.5μm easily reach the pulmonary alveoli and are intercepted by macrophages but if they are difficult to digest (e.g. crystalline silica) they could cause in time diseases of the lungs and surrounding organs (e.g. silicosis and mesothelioma). Infections like flu, TBC, hepatitis, HIV can be spread through contaminated aerosols [8].

A large part of the previous studies have used different particles collectors, with particles filters to estimate their concentration. The aim of our study was to determine more precise the size and nature of the dental aerosol particles and therefore we collected these particles on double-sided carbon adhesive tape which practically does not miss any element that reach them, after their exposure in the interest area. The use of this original collecting method and complex characterization techniques allowed to observe the presence of elements that have not been mentioned in previous studies. The results were correlated with those already reported in the literature in order to find if the identified particles could have toxic effects on humans.

2. Experimental

2.1 The sample collection and characterization

For collecting the samples, we used double-sided adhesive carbon tape of 5 mm × 2.5 mm and discs of 5mm diameter. It is necessary that both sides of the tape to be adhesive as a part adheres to the exposed area (clothing, medical mask) while on the outside will accumulate particles to be analysed.

Thus, the carbon tapes were attached to the dentist's, gloves, mask, protection shield, glasses or on the rubber dam of the patient during various treatments (ultrasound scaling, root canal preparation, cavity preparation and filling, removal of silver amalgam, composite, glass ionomer filings, teeth preparation for prosthetic purpose, orthodontic controls, etc.). A number of 35 samples were collected from 5 dentists with different specialties, with the consent of the patients.

2.2 The qualitative samples analysis in the laboratory

2.2.1 The scanning electronic microscopy (SEM) and energy dispersive X-ray analysis (EDX)

The samples were analysed by SEM to obtain data related to the shape, surface morphology and size distribution of the particles collected on the tape. SEM images where recorded with a Quanta 3D FEG SEM (FEI). The elemental composition of the samples was investigated using energy dispersive X-ray analysis (EDX) carried out on few micrometres in

depth of sample (2-5μm), not only on the outermost layer of the surface. The qualitative analysis of samples was carried out in 6 points for each sample. In order to amplify the secondary electrons signal the powders were metallised with a Pt-Pd thin layer of 5nm in a Q150R ES automatic Sputter Coater, in argon atmosphere.

2.2.2 The X – Ray photoelectron spectroscopy (XPS)

The elemental composition at the surface samples was analysed by XPS. The XPS measurements were performed by using a SPECS XPS instrument with a monochromatic Al Kα source (1486.69eV), operated with a power of 280W. Samples were mounted for spectroscopy directly on the sample holder using the double-sided adhesive carbon tape on which they were collected. The background pressure during measurements was 7×10^{-10}mbar. A low-energy charge neutralizer was used to remove the charge shifts during photoelectron emission. The binding energy (BE) scale was calibrated to that of the C 1s photoelectron peak (284.6eV). Survey scans were recorded at a pass energy Epass = 100eV, in steps of 1eV. Analysis of the data was carried out with Casa XPS software [9].

3. Results

3.1 Electronic microscopy analysis

SEM images of some selected samples (Fig. 1, 2) show evidence of the presence of a variety very small structures on the carbon tape, with different shapes and variable dimensions.

Some samples obtained during endodontic procedures are illustrated in figure 1. Figure 1a, recorded at a magnification of 1300x, depicts the wide range of particle sizes, from a few nanometres up to the micrometres range, present on the carbon tape. With increasing the magnification at 2000x (Fig. 1b), becomes more evident the variety of particles shapes, most of them with sharp edges that can produce lesions to the soft tissues they come in contact if they are inhaled. At higher magnification, 15000x, 10000x respectively (Fig. 1c,d) it can be observed that some of the samples may have retentive shapes that can easily bind other elements they reach, forming more complex systems that are less susceptible to be fagocitated.

Fig. 2 shows the electron microscopy characterization of some samples exposed during several procedures: teeth preparation for prosthetic purpose, endodontic treatment, orthodontic treatment and dental restoration. SEM images show the presence of structures on the carbon tape, with different shapes and variable dimensions (scale bar from 5 to 10 μm) which are a confirmation that no matter what the investigated treatment, the particles released as aerosols, by their size and shape, can represents pathogenicity factors for the human body.

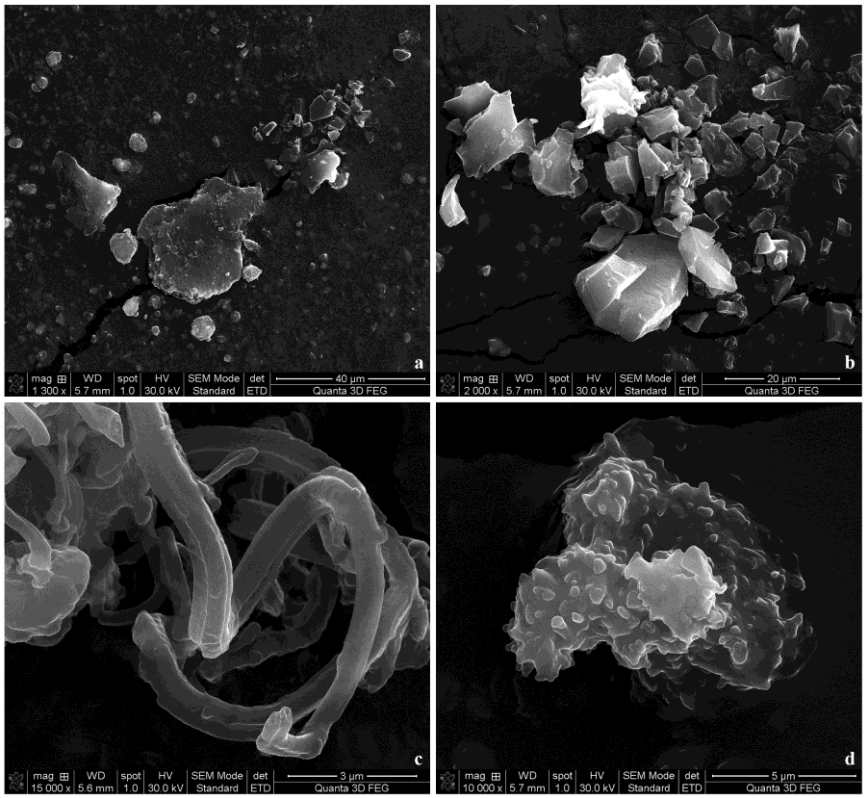

Fig.1. SEM characterization of some samples obtained during endodontic procedures. Images show the presence on the carbon tape of structures with different shapes and variable dimensions (scale bar from 3 to 40 μm)

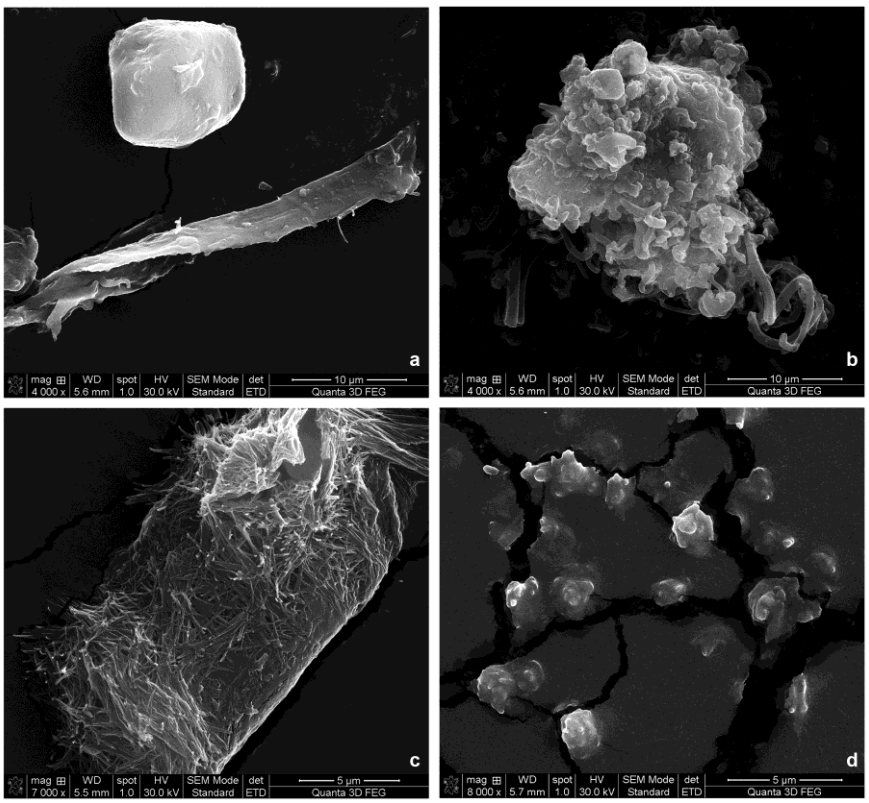

Fig. 2. Electron microscopy characterization of some samples exposed during: (a) teeth preparation for prosthetic purpose, (b) endodontic treatment, (c) orthodontic treatment, (d) dental restoration. SEM images show the presence of structures on the carbon tape, with different shapes and variable dimensions (scale bar from 5 to 10 μm)

The energy peaks in the EDX spectra correspond to various elements in the samples (see for exemplification figures 3b, 4b). Most samples contain C, O and Si. Depending on the type of the procedure other elements occur like: Ag, Al, Br, Ca, Ce, Cl, Cs, Cu, F, Fe, Fr, Hg, K, Lu, Mg, Mn, Mo, Na, Os, P, S, Sn, Sr, Ta, Ti, Tl, Tm, V, Zn. Silver amalgam fillings concentrated the largest number of compounds. By using the EDX system software one can analyse the energy spectrum in order to determine the abundance of the identified elements. The elemental compositions of 2 selected samples derived from EDX measurements are given in figure 3c, 4c in which the insets show the elements present in lower concentration.

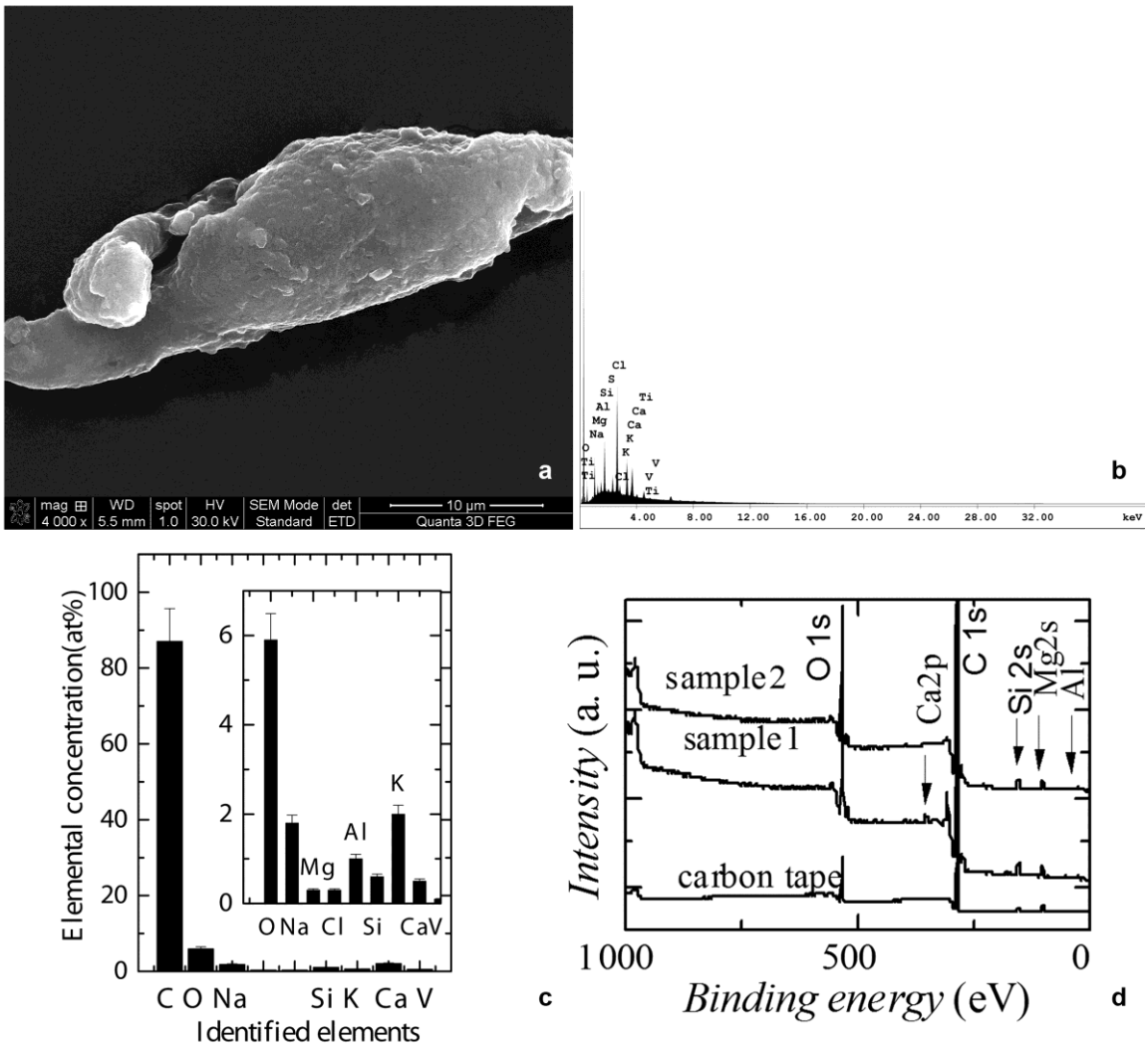

Fig. 3 Characterisation of the sample collected on the carbon tape during endodontic treatment with wear rotary instruments; SEM micrograph b) EDX spectrum; c) Elemental concentration (at%) derived from EDX; d) Sample surface composition derived from XPS measurements. Black arrows indicate the identified elements: O, C, Ca, Si, Mg, Al. The line at the bottom of the graphs represents the XPS spectra obtained on the carbon tape before its exposure in the dental office

3.2 Surface analysis results

Fig 3d and 4d show the XPS spectra obtained for three representative samples in the energy range 1100-0eV together with the corresponding spectrum obtained for the tape before its exposure in the dental office, in order to identify properly the characteristic spectral line of elements present on the carbon tape due to deposition of aerosols produced during various treatments. The recorded spectra indicate the elements present at the surface of the analysed

sample. These spectra are quantified in terms of line intensity and their position in the spectrum. The peak intensity is a measure of the amount of material on the sample surface while its position indicates the elemental and chemical composition. The results obtained are listed in Table 1.

Table 1. Compositions of three representative samples determined from XPS survey spectra

	Elemental Composition (at %)										
Sample	C 1s	Ca 2p	O 1s	Ta4f	Sn3d	Mg 2s	Al2p	Si 2p	Hg4f	Ag3d	N 1s
Sample 1	69,95	0,95	20,05	----	-----	2,33	-----	5,73	-------	------	0,96
Sample 2	77,8	-------	18,39	-----	-----	--------	-----	3,37	-------	-----	0,43
Sample 3	69.7	--------	18.7	0.2	1.2	--------	1.5	-------	0.8	0.6	7.1

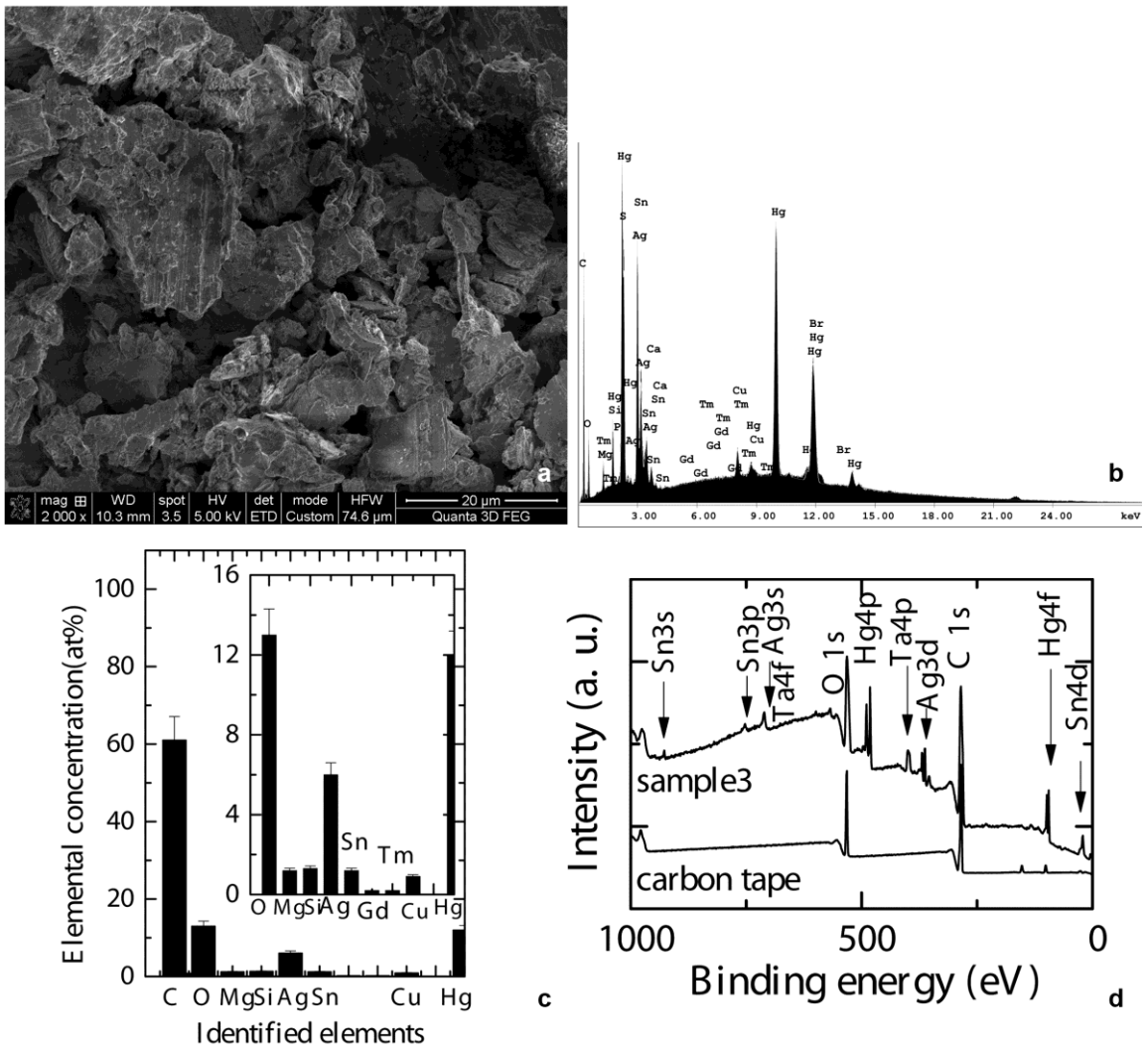

Fig. 4 Characterisation analysis of the sample collected during removal of silver amalgam filling; a) SEM micrograph b) EDX spectrum; c) Elemental concentration (at%) derived from EDX; d) Sample surface composition derived from XPS measurements. Black arrows indicate the identified elements: O, C, Si, Sn, Ag, Hg and Ta. The line at the bottom of the graphs represents the XPS spectrum obtained on the carbon tape before its exposure in the dental office

It was observed that spectra obtained by XPS method, in addition to the signal coming from the particles contained at the samples surface also contain signal from the carbon tape. This is because the carbon tape is not fully covered by the collected particles. The X-ray spot size on the

sample is 3.5x1mm^2 in the XPS technique, therefore it has been analysed an area larger than the size of the fragments located on the surface tape. However, the obtained XPS results confirm the presence on the samples outermost layer of the elements highlighted by EDX technique.

4. Discussion

Following the elemental analysis determined by EDX and XPS spectroscopy for different domains of the same sample, minor differences in the composition appeared, due to the fact that in the case of EDX the elemental analysis is carried out on few micrometres (approx. 5μm) in depth of sample, not only on the outermost layer of the surface as in the case of XPS [10]. The information provided by the EDX technique from a depth of several micrometres depends on several parameters such as: accelerating voltage, beam current, spot size, sample composition and homogeneity [11]. Moreover, the depth of electron beam penetration increases when the sample has a lower density. With a 40keV primary electron beam, the penetration depth is approx. 10μm. The depth at which the primary electrons have sufficient energy to generate characteristic X-rays is somewhat less than this, about half. Unlike SEM, XPS is a surface sensitive technique; the depth from where the signal is coming is approx. 0.005μm, with a detection limit of 1% in atomic concentration for chemical elements. Therefore elements with lower atomic concentration on sample surface (e.g. in our case V, Tm, Gd) cannot be detected by XPS [9, 10]. Table 1 illustrates that the elemental concentration determined at the sample surface varies from sample to sample depending on the exposure area and the type of the treatment carried out.

As a consequence, by using the EDX and XPS techniques we were able to identify both the presence of Hg and to determine particle size which contain it, as well as the presence of other elements: Cs, Fr, Lu, Mo, Os, Sn, Ta, Tl, Tm, V - all of which were not previously reported by similar studies.

In a previous study [7] using a X-ray fluorescence (XRF) equipment, Hg could not be detected; the study by Nimmo et al. [12] mentioned that when a high speed hand-piece is used to remove an amalgam restoration (from teeth placed in a typodont) the aerosols carry amalgam particles smaller than 10μm, containing Hg. Regarding the source of various elements detected here by EDX and XPS, recent studies have shown that P, Ca, Si, Ba and Sr besides the direct dental origin may derive from glass ionomer cements and composite materials [13]. It has been reported by another group [14] that the tooth enamel was contaminated with following elements: Cu, Fe, Mg, Mn, Pb, Sr and Zn, which can be released also from preparing the tooth enamel. If some of the compounds such as particles from dental tissue, bone, hydroxyapatite, wax, acrylic, silicone are more easily digested by pulmonary macrophages, which can thus be removed, particles of SiO_2 (silica crystals), stainless steel, alloys of Au, Titan, Cr-Co, Zr, ceramics are more difficult to disintegrate [8].

Muller [15] reported that nanoparticles (NPs) can cause bronchial and lungs inflammation, pulmonary fibrosis, bronchial asthma exacerbation if this was already present, favouring allergic reaction type I, show carcinogenic potential depending on the chemical structure and not the least effects on the cardiovascular apparatus and other organs.

During the removal of Ag amalgam fillings we identified elements with moderate to high toxicity: Ce, Cs, Gd, Hg, La, Lu, Os, Tl and we found Fr and V during the treatment of root channels with rotary instruments, handpiece and root instruments made of NiTi. Likewise, different metals are released from the burs used during different treatments or even from the head of handpieces, where the bur is fixed [3, 4]. Thus it becomes evident the necessity to eliminate of use the wear instruments because it can release elements with toxicity for the human body.

Hg is treated by World Health Organisation as a hazard in the workplace and occupational exposure limits were established [16]. Controlled studies have described severe effects at workers with chronic exposure to mercury vapours, even at low concentrations of 0.7-42μg/m^3 [17]. Amalgam fillings are subject to wear and corrode over time. Vimy et al. [18] analysed the oral cavity air from patients with amalgam fillings and found that it contains Hg vapour whose concentration increases after chewing and therefore these patients have a major source of chronic exposure to Hg. The effects of mercury vapour inhalation are mainly on central and peripheral

nervous system and kidneys. Environmental Protection Agency in 2001 lowered the daily dose considered safe in 1978 from 0.5μg/kg body weight /day to 0,1μg/kg body weight/day [19]. Hg analysis in urine is suitable to confirm Hg exposure due to amalgam fillings, while hair mercury better reflects Hg intake by fish consumption [20]. Moreover, there is a relationship between maternal amalgam fillings and prenatal exposure to Hg [21].

Thallium is used in the glass industry but also in alloys with Ag, being one of the ingredients of the amalgam fillings having the role in adhesion to the tooth surface [22]. It is a very toxic element to humans, more toxic than Hg, Cd, Pb, Cu and Zn, and a relationship between maternal amalgam fillings and prenatal exposure to Hg, absorbed through the skin and mucous membranes (accumulating mainly in bones, kidneys and central nervous system) was established [23]. The compounds of thallium are highly toxic and lead to changes in cell-cycle progression [24]. Also vanadium compounds should be considered toxic, especially inorganic salts. Toxic effects have been reported mainly in the kidney and liver [25]. Vanadium is used as ferrovanadium or as an additive in steel and is used in high speed tool steel and surgical instruments.

Furthermore, it became apparent that there is an impressive heterogeneity of the generic called “silver amalgam”. We found in four different fillings that we have removed, elements that were not present in the others similar fillings. Since materials to be applied are subject to continuous corrosion in oral environment, to avoid them in case of patient allergies or intolerances to some of their compounds it comes off the need for a unitary recipe, known by both, physician and patient, in order each of them to be aware of the risks. Dental composites contain up to 60 vol% nanosized fillers which release airborne NPs in the breathing zone of dentist and patient [2]. Different types of NPs present specific physico-chemical characteristics and their potential adverse impact on human health must be always investigated [26]. Recent studies indicate that nanosized metals (e.g., Ni, Co, V) represent risk factors for lung diseases, because many of these have fibrogenic or carcinogenic effects in humans [27] and confirm that the key mechanism of nontoxicity is the biological oxidative damage (BOD) [28]. An extended review on the potential risk of nanomaterials sustains that particle size and surface area are important particle parameters, in relation to hazards, and that there is a central nervous system effect by transsynaptic transport after inhalation, through the olfactory epithelium and uptake through the blood-brain barrier [29]. A 2013 review which attempted to detect oxidative DNA damage in humans, laboratory animals, and cell lines states that the main factors that determine the toxicological effects of NPs in the body are the characteristics of the exposure (e.g., penetration route, duration, and concentration) and of the exposed organism and the intrinsic toxicity of NPs [30].

Some authors insist on the importance of a unitary protocol of characterization for NPs so that the results can be comparable and the properties of particles be fully characterized before the in vitro studies [31, 32, 33]. We would add that because they already act on the human beings, the in vivo studies are urgent to be completed.

As means to reduce the contamination, in addition to the usual gloves and face mask, data from the literature highlight the usefulness of the rubber dam, a high volume evacuator and rinsing with antiseptic solution before starting dental treatment (e.g. Chlorhexidine 0.01 %). It is also recommended the use of protection face shield and of an adequate ventilation and air filtration in the dental office to minimize the risk of chronic respiratory diseases over time. In Japan the Aeroservice Company has developed a product Aerosystem 35 that can filter particles with sizes up to 10nm [8].

5. Conclusions

Our study shows that due to the successfully combination of the methods that we described here we were able to determine very precise the size and nature of the dental aerosol particles collected after their exposure in the interest area of dental offices. These methods complete each other very well, in terms of the different depth from which the signal is analysed. Therefore the use of these methods allowed to identify elements that have not been mentioned in previous studies.

This work highlights the wide variety of substances handled inside a dental office, many of which with pathogenic potential, especially through slow accumulation over time. The results obtained in this study draw the attention to the occupational risks in the dental medicine field and the importance of protective measures for both doctor and patient. Some of the elements detected in the samples have been studied mainly on animals, therefore are very few clinical studies on humans, which requires further research in this direction because, as seen in the case of Hg, over time, the maximum doses that were initially considered safe, became much lower. Thus an adequate combination of investigation techniques may lead to a better understanding of the toxicity mechanisms for improving the management of the procedures responsible for highly toxic aerosols. The characteristics of aerosols released from different dental materials and dental instruments during their use in the oral cavity impose the necessity for the manufactures of a careful choose of the materials that compose a final product.

Acknowledgements

This paper was published under the frame of European Social Found, Human Resources Development Operational Programme 2007-2013, project no. POSDRU/159/1.5/S/138776.

References

[1] S. K. Harrel, J. Molinari, J Am Dent Assoc., **135**(4), 429 (2004).
[2] K. L. Van Landuyt, B. Hellack, B. Van Meerbeek, M. Peumans, P. Hoet, M. Wiemann et al. Acta Biomater, **10**(1), 365 (2014).
[3] C. J. Day, R. Price, J. R. Sandy, A. J. Ireland, Am J Orthod Dentofacial Orthop, **133**(1), 11 (2008).
[4] A. J. Ireland, T. Moreno, R. Price, Am J Orthod Dentofacial Orthop, **124**, 683 (2003).
[5] P. A. Leggat, U. Kedjarune, Int Dent J, **51**, 39 (2001).
[6] F. Ballester, S. Medina, E. Boldo, P. Goodman, M. Neuberger, C. Iñiguez et al, J Epidemiol Community Health, **62**(2), 98 (2008).
[7] M. Sotiriou, S. F. Ferguson, M. Davey, J.M. Wolfson, P. Demokritou, J. Lawrence et al. Environ Monit Assess, **137**(1-3), 351 (2008).
[8] M. Taira, M. Sasaki, S. Kimura, Y. Araki, Nano Biomedicine, **1**(1), 9 (2009).
[9] N. Fairley, A. Carrick, The Casa Cookbook - Part 1: Recipes for XPS Data Processing, Acolyte Science, Cheshire, UK (2005).
[10] J. F. Watts, J. Wolstenholme, An introduction to surface analysis by XPS and AES. pp. 224. Wiley-VCH (2003).
[11] J. Goldstein, D. E. Newbury, D. C. Joy, C. E. Lyman, P. Echlin, E. Lifshin et al, Scanning electron microscopy and X-ray microanalysis, Springer, (2003).
[12] A. Nimmo, M. S. Werley, J. S. Martin, M. F. Tansy, J Prosthet Dent **63**(2), 228 (1990).
[13] M. Zhou, J. L. Drummond, L. Hanley, Dent Mater, **21**, 145 (2005).
[14] E. Reitznerová, D. Amarasiriwardena, M. Kopčáková, R. M. Barnes, Fresenius J Anal Chem, **367**(8), 748 (2000).
[15] M. Müller, M. Fritz, A. Buchter , Nanotoxikologie, Zbl Arbeitsmed, **58**, 238 (2008).
[16] WHO, Future use of materials for dental restoration, Report of the meeting convened at WHO HQ, Geneva, Switzerland (2011).
[17] C. H. Ngim, S. C. Foo, K. W. Boey, J. Jeyaratnam, Br J Ind Med, **49**(11), 782 (1992).
[18] M. J. Vimy, F. L. Lorscheider, J Dent Res, **64**(8), 1069 (1985).
[19] T. W. Clarkson, L. Magos, G.J. Myers, N Engl J Med, **349**(18), 1731 (2003).
[20] A. Pesch, M. Wilhelm, J Expo Anal Environ Epidemiol, **12**(4), 252 (2002).
[21] L. Palkovicova, M. Ursinyova, V. Masanova, Z. Yu, I. Hertz-Picciotto, J Expo Sci Environ Epidemiol, **18**(3), 326 (2008).
[22] R. F. Harvey, Dental amalgam U.S. Patent No. 3,554,738 (1971).
[23] A. L. Peter, T. Viraraghavan, Environ Int, **31**(4), 493 (2005).

[24] J. J. Rodríguez-Mercado, M. A. Altamirano-Lozano, Drug Chem Toxicol, **36**(3), 369 (2013).
[25] A. K. Srivastava, M. Z. Mehdi, Diabet Med, **22**(1), 2 (2005).
[26] A. Pandey, A. K. Singh, S. K. Maurya, R. Ra, H. S. Shukla, Dig J Nanomater Biostruct, **3**(3), 141 (2008).
[27] J. C. Bonner, Proceedings of the American Thoracic Society, **7**(2), 138 (2010).
[28] S. F. Hsieh, D. Bello, D. F. Schmidt, A. K. Pal, A. Stella, J. A. Isaacs et al, Small, **9**(9-10), 1853 (2013).
[29] P. J. Borm, D. Robbins, S. Haubold, T. Kuhlbusch, H. Fissan, K. Donaldson et al, Part Fibre Toxicol, **3**(1), 11 (2006).
[30] K. T. Rim, S. W. Song, H. Y. Kim, Saf Health Work, **4**(4), 177 (2013).
[31] D. B. Warheit, Toxicol Sci, **101**(2), 183 (2008).
[32] C. F. Jones, D. W. Grainger, Adv Drug Deliv Rev, **61**(6), 438 (2009).
[33] T. Kuhlbusch, C. Asbach, H. Fissan, D. Göhler, M. Stintz, Part Fibre Toxicol, **8**(1), 22 (2011).