INVESTIGAREA EXPERIMENTALĂ AVANSATĂ A UNOR BIOMATERIALE METALICE FOLOSITE
ADVANCED EXPERIMENTAL INVESTIGATION OF USED METALLIC BIOMATERIALS

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The paper aims to present the investigation of metallic biomaterials by using state of the art investigation techniques and instruments in order to assess their degradation after they have been used as implants. Thus, several metallic biomaterials used for different periods of time by different patients were collected and analysed by functional and structural point of view, meaning that mechanical properties will be determined and the functionality of the metallic biomaterials will be assessed and structural point of view. Metallic biomaterials consisted of: one dental Cr-Co based material used for 6 months, one dental Ni-Cr based material used for 5 years and one Fe-Cr based orthopaedic screw used for 3 months. It is to be mentioned that the exact composition of the biomaterials before they were used as implants was unknown. The functional investigation of the metallic biomaterials consisted of hardness measurements by using a Vickers micro hardness tester, the structural investigation of the materials consisted of light and electronic microscopy by using an FEI INSPECT F electronic microscope coupled with X-Ray diffraction EDAX type and composition determination by using an ICP-MS spectrometer.

Lucrarea își propune să prezinte investigarea unor biomateriale metalice folosind tehnici și aparatură de investigație avansate cu scopul de a se stabili în ce măsură s-au degradat după ce au fost folosite ca implanturi. Astfel, mai multe probe diferite de biomateriale metalice folosite pe perioade diferite de timp și de către pacienți diferiți au fost prelevate și investigate din punct de vedere funcțional și structural. Biomaterialele metalice prelevate au constat în: un material dentar pe bază de aliaj Co-Cr folosit timp de 6 luni, un material dentar pe bază de Ni-Cr folosit timp de 5 ani și un șurub ortopedic pe bază de Fe-Cr folosit timp de 3 luni. Este de menționat că nu se cunoaște compoziția exactă a biomaterialelor înainte de a fi implantate. Investigarea funcțională a biomaterialelor metalice a constat în determinarea durității probei folosind un microdurimetru Vickers, iar microstructura a fost determinată prin microscopie optică folosind un microscop FEI INSPECT F dotat cu analizor de raze X tip EDAX și determinarea compoziției prin spectroscopie ICP-MS.

Keywords: *metallic biomaterials, hardness, mass spectroscopy, light microscopy, electronic microscopy.*

1. Introduction

Cr based materials are biocompatible [1,2] and are wide used as orthopaedic and dental implant materials in clinical practice such as hip joint, knee replacement and as dental alternates due to their superior mechanical properties, good wear and corrosion resistance [3]. The biocompatibility of Co-Cr based materials is closely related to its excellent corrosion resistance due to the presence of extremely thin passive oxide film that spontaneously forms on the alloy surface [3]. XPS analysis reveals that its composition is predominantly Cr_2O_3 oxide with minor contribution from Co and Mo oxides [4, 5]. These films also form on the surface of other metallic biomaterials and serve as a barrier to corrosion processes in alloy systems that would otherwise experience very high corrosion rates [6].

Metal ions from implants are released into surrounding tissue by various processes, including corrosion, wear and mechanically accelerated processes such as stress corrosion, corrosion fatigue and fretting corrosion. Research shows that ion metal release is associated with clinical implant failure, osteolysis, skin allergies and remote site accumulations [7].

It was found that the surface oxide film of Cr alloys inhibits the dissolution of metal ions but is not always stable in the human body. Hanawa et al. [8] characterized the surface oxide films formed on Cr alloys during immersion in various biological environments [8].

Due to the fact that the quantification in time of the amount of ion release in a specific media is related to the corrosion rate within such media, the present research is focused on the determination of metallic alloy corrosion by using advanced

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experimental investigation [9]. Taking into account that even a small amount of metallic ion release into the body could be very aggressive due to their migration and accumulation in different organs, sometimes far away from the point of release, it is very important to quantify the amount of ion release in various environments, thus, ICP-MS investigations were conducted [10].

The work aims to evaluate the metallic biomaterials by electronic microscopy coupled with X-Ray energy dispersive analyzer SEM-EDS, light microscopy and inductively coupled plasma mass spectroscopy ICP-MS [11] for the following samples: sample 1-dental Cr-Co based material used for 6 months, sample 2-dental Ni-Cr based material used for 5 years and sample 3-Ni-Cr based orthopaedic screw used for 3 months.

2. Experimental

While most of the scientific papers are reporting metallic biomaterials characterization by assessing their corrosion in different body fluids, the present paper focuses on material characterization after they have already been used in the human body. This approach on metallic biomaterials is a novelty within biomaterial research and is the most suitable one to assess the biomaterial degradation within the human body after some usage periods.

The metallic biomaterials samples were used implants which were removed from the patients and instead of being destroyed were used for research purposes. They were metallic pieces and all the data related to them was the time of usage and a roughly description of each material; it was only known the major metal in their structure, as: Co-Cr, Ni-Cr, Fe-Cr. Each of them was prepared for the specific analysis as follows: for optic and electronic microscopy, the samples were polished and their surface was treated with specific acid mixture in order to enhance metallic phases. After the microscopy analysis was performed, the samples were then tested for micro-hardness and, in the end, by using a diamond rasp some fine grinding was performed in order to obtain small grains for a better digestion within the acids. The obtained solution was then used for ICP-MS analysis.

2.1. Method for light microscopy analysis

Light microscopy in materials analysis generally refers to reflected light microscopy. In this method, light is directed vertically through the microscope objective and reflected back through the objective to an eyepiece, view screen, or camera. Transmitted light is occasionally used for transparent and translucent materials. For some low-magnification work (stereo microscopy), external, oblique illumination can be reflected off the sample into the objective.

Magnification of the sample image is obtained by light refraction through a combination of objective lenses and eyepieces. The minimum feature resolution is approximately 0.2 μm. However, smaller features - as small as about 0.05 μm - can be detected by image contrast enhancement with polarized light, interference contrast, and dark field illuminations. The resulting images can be recorded either on traditional films or as digital files for computer display, analysis, and storage [12].

2.2. Method for SEM-EDS analysis

Energy Dispersive X-Ray Spectroscopy (EDS or EDX) is a chemical microanalysis technique used in conjunction with scanning electron microscopy (SEM). The EDS technique detects X-rays emitted from the sample during bombardment by an electron beam to characterize the elemental composition of the analyzed volume. Features or phases as small as 1 μm or less can be analyzed.

When the sample is bombarded by the SEM's electron beam, electrons are ejected from the atoms comprising the sample's surface. The resulting electron vacancies are filled by electrons from a higher state, and an X-ray is emitted to balance the energy difference between the two electrons' states. The X-ray energy is characteristic of the element from which it was emitted.

The EDS x-ray detector measures the relative abundance of emitted X-rays versus their energy. The detector is typically a lithium-drifted silicon, solid-state device. When an incident X-ray strikes the detector, it creates a charge pulse that is proportional to the energy of the x-ray. The charge pulse is converted to a voltage pulse (which remains proportional to the X-ray energy) by a charge-sensitive preamplifier. The signal is then sent to a multichannel analyzer where the pulses are sorted by voltage. The energy, as determined from the voltage measurement, for each incident X-ray is sent to a computer for display and further data evaluation. The spectrum of X-ray energy versus counts is evaluated to determine the elemental composition of the sampled volume.

The samples were embedded in resin and they been polished by using polishing paper with grain size ranging from 120 to 1200 μm and then polished by using a 1 μm diamond like suspension and held until it looked like a mirror. Sample's surfaces have been chemically treated by using a chemical reagent mixture made of: 10 ml HNO_3 + 10 ml CH_3COOH + 15 ml HCl + 1 ml glycerine. This treatment was made in order to emphasize different metallic polytypes and the reagent is specific to Cr based metallic alloys.

2.3. Method for microhardness analysis

Micro-hardness testing (or micro-indentation hardness testing) is a method for measuring the hardness of a material on a microscopic scale. A precision diamond indenter is impressed into the material at loads from a few grams to 1 kilogram. The impression length, microscopically measured and the test load are used to calculate a hardness value. The hardness values obtained are useful indicators of a material's properties and expected service behaviour. Conversions from micro-indentation hardness values to tensile strength and other hardness scales (e.g., Rockwell) are available for many metals and alloys.

The indentations are typically made using either a square-based pyramid indenter - as in the present paper, (Vickers hardness scale) or an elongated, rhombohedral - shaped indenter (Knoop hardness scale). The tester applies the selected test load using dead weights. The length of the hardness impressions are precisely measured with a light microscope using either a filer eyepiece or a video image and computer software. A hardness number is then calculated using the test load, the impression length, and a shape factor for the indenter type used for the test.

2.4. Method for ICP-MS analysis

Inductively coupled plasma mass spectrometer is a powerful method for determining metal traces within given environments due to its capability to measure very low concentrations of metallic ions dissolved within fluids. This approach is well known, widely used by various research teams and reported in high quoted scientific journals.

ICP-MS is a multi-element technique characterized by high selectivity, sensitivity and detection limits much lower than other multi-element techniques. Inductively coupled plasma mass spectrometry (ICP-MS) together with microwave digestion is considered an excellent tool for detailed characterization of the elementary composition of many samples [13].

ICP-MS prove detection limits at ng L^{-1} level for many elements. The linear dynamic range of 4 to 5 orders of magnitude and the ability to do multi-element analysis are also excellent analytical features for ICP-MS. The direct determination of major constituents by ICP-MS is often difficult because the analytes with concentrations above 1 mg L^{-1} cause the saturation of the detector. Thus, when it is required to determine both major and trace elements, the major constituents must be directly determined by ICP-AES or by ICP-MS after sample solution dilution and then the trace elements are determined by ICP-MS. Such separate measurements are time consuming. In addition, the separate determination of major and trace elements presents difficulties when the analysis is done with limited sample amounts [14].

But the most challenging shortcoming of ICP-MS is the existence of non-spectroscopic and spectroscopic interferences for some elements. In order to reduce or to eliminate the matrix interferences, calibration standards or blanks should be prepared in the same matrix as the samples. Also, dilution factors in the range of 50–500 may assist to minimize the influence of the sample to matrix effects. Argon background interference and oxygen-, nitrogen-, and hydrogen-containing interferences are significant because of their constant introduction to the system. In some cases, these interferences can be avoided by optimal selection of analytical isotope [15].

Nevertheless, the use of ICP-MS method to determine the composition of an unknown metallic biomaterial has not been used so far.

Basically, ICP-MS method consists in determining the amount of metallic ions within a given solution. The first step is to transform the metal into a liquid containing its ions. This procedure is called "digestion" and consists of transforming the metal rasping into ion solution under the influence of very concentrated acid mixture, pressure and heating.

3. Sample Preparation

Thus, samples were prepared for analysis by closed-vessel microwave-assisted digestion. Sample masses consisting in 100 mg of metal rasping were measured gravimetrically at a precision of ±0.05 mg. The digestion solution comprised 1 mL of concentrated HNO_3, 5 mL of HCl 37% and 2 ml of 30% H_2O_2 was added to enhance oxidative degradation processes. The vessels sit uncapped for 30 minutes to allow for any pre-reactions to occur safely before being capped and digested following the program.

The digestion program consisted of two steps:

- heating the sample at 200^0C and 35 bar of pressure at a power rate of 90% in 10 minutes and maintaining these parameters for 30 minutes, and
- decreasing the temperature to 50^0C and the pressure to 25 bar in 1 minute and maintaining these parameters for 10 minutes

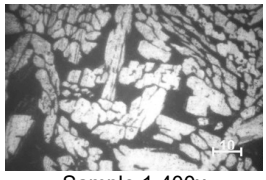
Then, after reaching room temperature, the vessels were carefully opened avoiding the fume and vapours contact with skin and/or eyes [16].

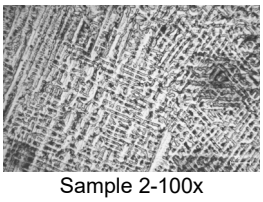
The reagent blank consisting in the same mixture of acids as for the samples has been digested along with the metallic rasping in order to be used as reference for metal measurements.

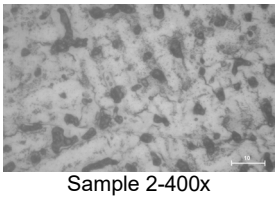
4. Standard Preparation

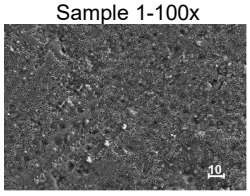
Five standard concentrations and a blank solution were used to prepare the calibration curve for each element. The calibration standards ranging from 0.1 to 10 µg/mL were prepared


Sample 1-100x


Sample 1-400x


Sample 2-100x


Sample 2-400x


Sample 3-100x

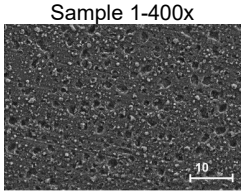

Sample 3-400x

Fig. 1 - Optical microscopy of the samples at 100x and 400x magnification / *Microscopia optică realizată pe probe la o magnificație de 100x și 400x.*

by diluting the 100 µg/mL multi-element stock standard with 2.5 mL HCl 37%, 0.5 mL HNO_3 65% and deionized water to 25 mL volume.

5. Results and discussions

5.1. Characterization of the materials

Each metallic biomaterial was characterized both from structural and functional point of view. Structural characterization was made by: light microscopy, SEM-EDS electronic microscopy and composition determination by ICP-MS. Functional characterization was made by Vickers hardness determination.

5.2. Structural characterization by light microscopy

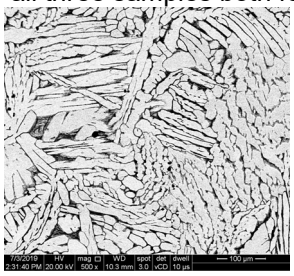
The method is based on visual analysis of the sample in order to assess the microstructure of the metallic alloy. The sample was polished with the smallest size of the polishing powder and then was placed under the microscope objective. The magnification used was: 100x and 400x.

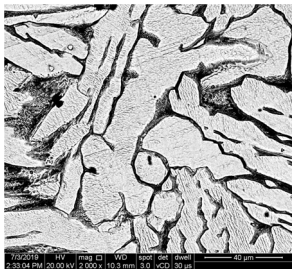
In Figure 1 are presented the optical images for all three samples both for 100x and 400x.

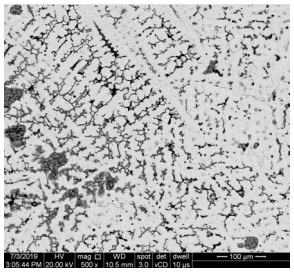
As it can be observed in Figure 1, all three samples have a well-defined homogenous structure with minor inter-grain pores making them mechanically resistant and lowering the possibility to form oxide layers at the surface of each individual grain. This characteristic ensures that the metallic biomaterial does not corrode very soon after its implantation within the body. Also the homogenous structure of the material ensures its mechanical resistance.

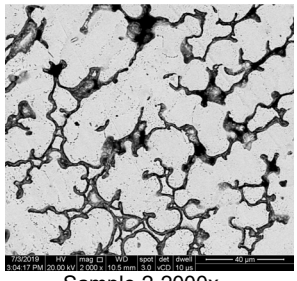
5.3. Structural characterization by SEM-EDS microscopy

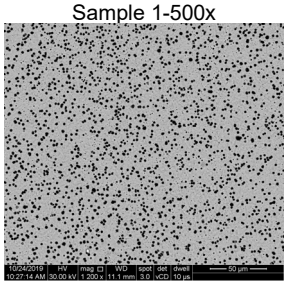
The method is based on the detection of X-rays emitted from the sample during bombardment by an electron beam to characterize the elemental composition of the analyzed volume. During the analysis, the magnification of the sample was modified and the structural composition was assessed. This technique is a very versatile one due to its capabilities to give both qualitative and quantitative informations regarding the microcrystalline structure of the sample. Also, along with assessing the structural composition of the samples, SEM images have been collected in order to assess their internal structure.

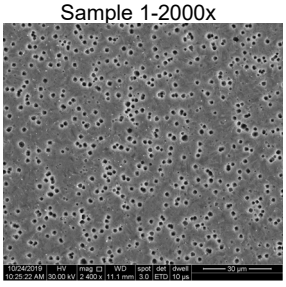

Sample 1-500x

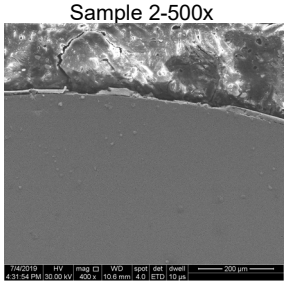

Sample 1-2000x

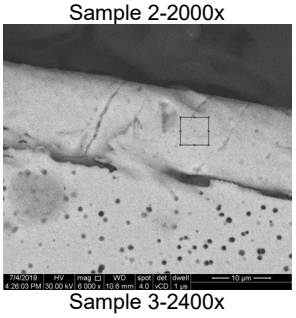

Sample 2-500x


Sample 2-2000x


Sample 3 - 1200x


Sample 3-2400x


Sample 3 - 1200x


Sample 3-2400x

Core Oxide layer

Fig. 2 - SEM images of the samples / *Imaginile SEM ale probelor*

As it can be seen in Figure 2, SEM analysis of the samples show the microstructure of them.

The dental materials show a very well defined grains, which are elongated and have inter-grain delimitations due to the metal preparation. Even though, the metal is compact and show no fractures of any kind. The materials used as dental implants does not present any corrosion and also no oxide film was formed on their surface.

Regarding sample 3, the orthopaedic screw is actually an austenitic alloy, which has no magnetic properties. The inner structure is homogenous presenting just some pits as resulted from the material preparation. The orthopaedic screw also presents an oxide layer on its surface, thus increasing its corrosion resistance since, unlike the dental materials which have contact mainly with human saliva, the orthopaedic screw has contact with blood and lymph having a higher probability to dissociate metallic ions into blood stream. The formed oxide layer is continuous and has no gaps, pits and/or fractures.

EDS technique has been applied to the samples in order to assess their qualitative and quantitative composition.

Table 1

Chemical composition of sample 1 based on EDS spectrum
Compoziția chimică a probei 1 pe baza spectrului EDS

Element	SiK	MoL	CrK	NiK
Weight % by Element	2.70	12.86	24.34	60.10
Atomic % by Element	5.58	7.79	27.18	59.45

As it can be observed in Figure 3 and Table 1, the chemical composition of sample 1 has been determined and it showed a Ni-Cr based alloy, that has traces of Mo and Si. Ni-Cr dental alloys are well known to have a long lifespan and a good mechanical resistance, and by coupling this analysis with SEM microscopy it is clearly shown that the material is corrosion resistant.

Figure 4 and Table 2 summarize the chemical composition of sample 2. Unlike sample 1, this sample has Mn in its internal structure. The presence of Mn within this sample leads to a different internal structure as it can be seen in optical microscopy analysis which is more fluid with the grains having more "rounded" shapes. This structure increases the mechanical resistance of the material being used for biomaterial that can withstand more elevated forces (mainly molars).

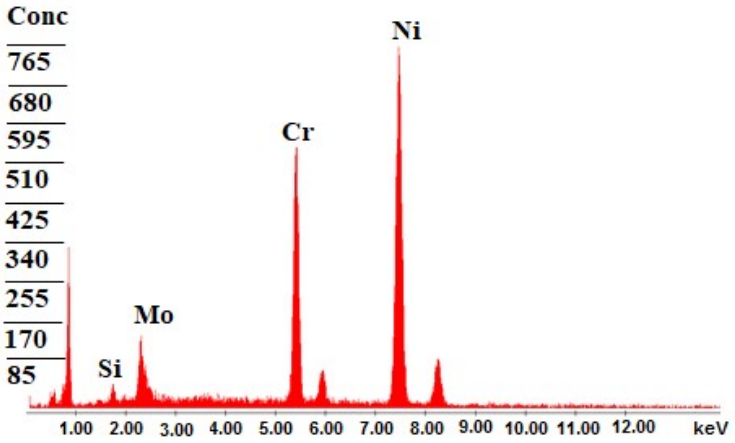


Fig. 3 - EDS spectra for sample 1 / *Spectrul EDS al probei 1*

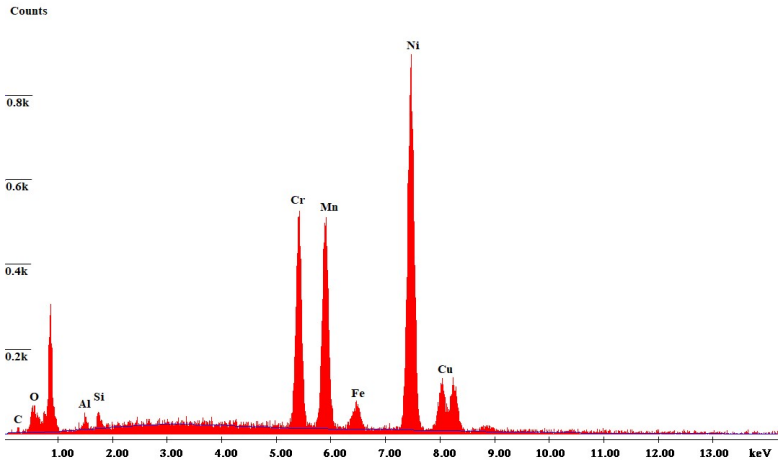


Fig. 4 - EDS spectra for sample 2 / *Spectrul EDS al probei 2*

Table 2

Chemical composition of sample 2 based on EDS spectrum
Compoziția chimică a probei 2 pe baza spectrului EDS

Element	C K	O K	AlK	SiK	CrK	MnK	FeK	NiK	CuK
Weight % by Element	2.47	2.55	2.40	2.10	15.03	16.65	2.50	47.59	8.71
Atomic % by Element	9.73	7.55	4.20	3.53	13.68	14.34	2.12	38.36	6.48

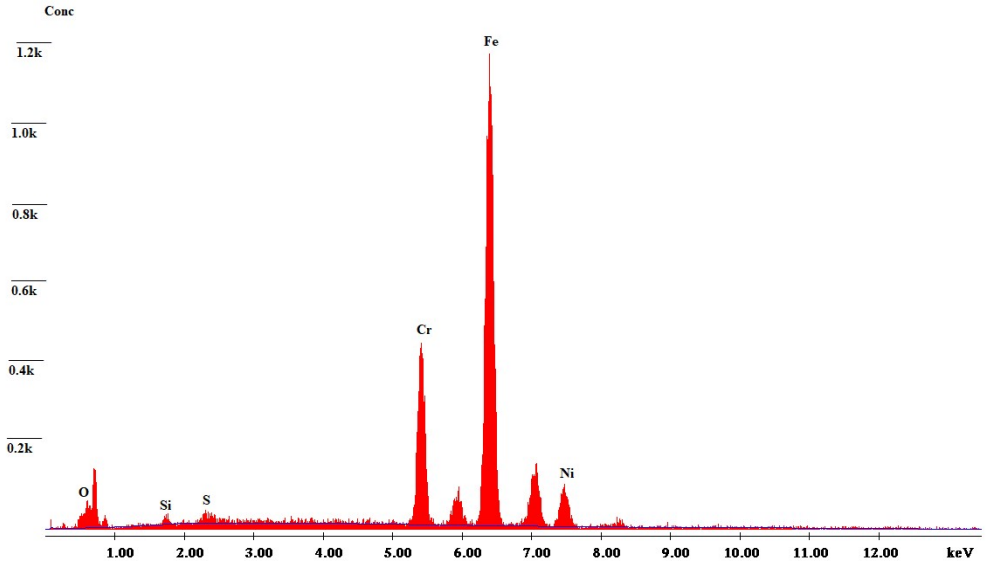


Fig. 5 - EDS spectra for sample 3 – core / *Spectrul EDS al probei 3 – secțiune*

Table 3

Chemical composition of sample 3 - core based on EDS spectrum
Compoziția chimică a probei 3 pe baza spectrului EDS - secțiune

Element	C K	O K	SiK	S K	CrK	FeK	NiK
Weight % by Element	2.02	1.86	1.63	1.79	16.5	66.35	9.85
Atomic % by Element	8.12	5.61	2.8	2.7	15.32	57.35	8.1

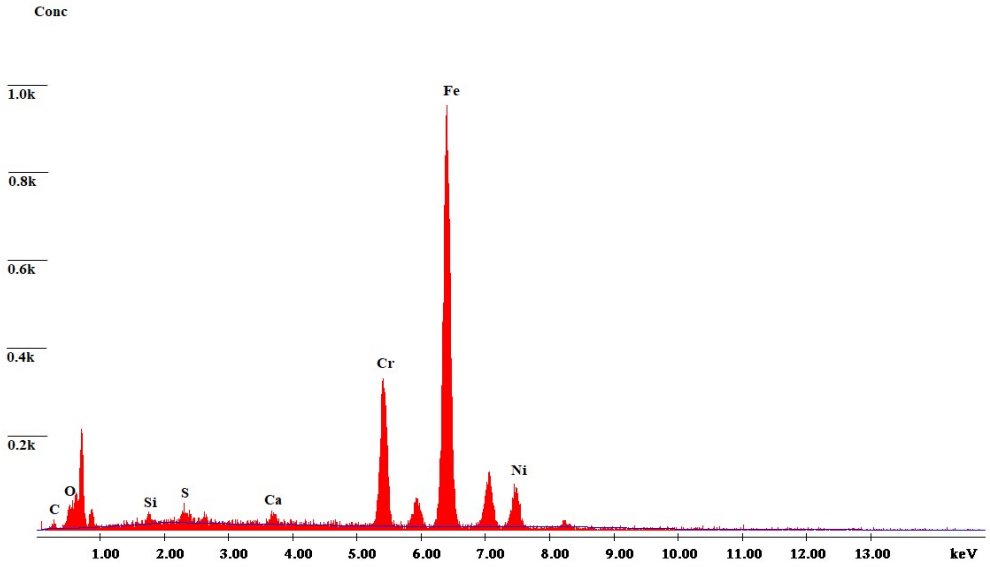


Fig. 6 - EDS spectra for sample 3 – layer / *Spectrul EDS al probei 3 – pe suprafață*

Table 4

Chemical composition of sample 3 - layer based on EDS spectrum
Compoziția chimică a probei 3 pe baza spectrului EDS – pe suprafață

Element	O K	SiK	S K	CrK	FeK	NiK
Weight % by Element	1.9	1.8	1.84	17.16	67.2	10.09
Atomic % by Element	6.12	3.29	2.94	16.96	61.85	8.83

Table 5

Chemical composition of the samples by ICP-MS
Compoziția chimică a probelor înregistrată prin ICP-MS

Element	Meas.	Sample 1 - Mean	Sample 2 - Mean	Sample 3 - Mean
Cr (mg/L)	1	2.721	1.757	1.892
Ni (mg/L)	2	7.828	8.378	1.412
Fe (mg/L)	3	-	0.049	6.331

Table 6

Microhardness values / *Valorile microdurității*

Sample 1	Microhardness HV 0.3			Mean
	250	252	246	249.3
Sample 2	Microhardness HV 0.3			Mean
	224	239	233	232
Sample 3	Microhardness HV 0.3			Mean
	247	252	251	250

Figure 5 and Table 3 are showing the chemical composition of the core of the orthopaedic screw. As it can be seen the material is Fe-Cr based having some Ni traces. Figure 6 and Table 4 are showing the chemical composition of the oxide layer that is covering the screw.

By comparing the two data sets, it can be noticed that Cr weight % concentration in the oxide layer is 4% higher than in the core, Fe concentration is 1% higher and Ni is 2,5% higher in the layer, so the oxide layer is mainly formed by Cr_2O_3 and NiO. Since the oxide layer of the orthopaedic screw does not present any pitting or other form of corrosion it is natural to assume that by forming the oxide layer, the corrosion resistance of the material increased.

5.4. Compositional characterization by ICP-MS analysis

ICP-MS is a type of mass spectrometry that uses an Inductively coupled plasma to ionize the sample. It atomizes the sample and creates atomic and small polyatomic ions, which are then detected. It is known and used for its ability to detect metals and several non-metals in liquid samples at very low concentrations. It can detect different isotopes of the same element, which makes it a versatile tool in Isotopic labelling.

Compared to atomic absorption spectroscopy, ICP-MS has greater speed, precision, and sensitivity. However, compared with other types of mass spectrometry, such as thermal ionization mass spectrometry (TIMS) and glow discharge mass spectrometry (GD-MS), ICP-MS introduces many interfering species: argon from the plasma, component gases of air that leak through the cone orifices, and contamination from glassware and the cones.

Since the metal concentrations within the samples are high, the samples were diluted in order not to affect the sensibility of the spectrometer and for the measurement results to fit the calibration curve. The calculated values for the metal concentrations are shown in Table 4.

As it can be observed in Table 5, the amount of metal within the samples varies accordingly to the use of the biomaterial. Thus, the values are comparable with the ones obtained by using alternative methods as EDS with only the observation that, in this case of ICP-MS analysis, Fe concentration measurement can be influenced by the Ar ions within the plasma.

5.5. Functional characterization by microhardness analysis

Microindentation hardness testing was performed on samples that have been metallographically mounted and polished. These samples were no larger than about 1 in. (25 mm) by 1 in. (25 mm) by 1/2 in. (12 mm) thick. The surface finish was a high-quality metallographic polish.

It is to be observed that all the samples present microhardness values (table 6) within the range for this kind of metallic alloys and their use as biomaterials did not influence their functional characteristics.

6. Conclusions

- All three metallic biomaterials that have been analysed both functional and structural present typical metallic biomaterials feature in the terms of microscopic structure, chemical composition and mechanical properties.
- Metallic biomaterial characterization emphasizes that none of them present any corrosion (as pits, gaps, oxidation, etc.) and/or structural failure (as bending or fracture), so it can be concluded that these materials have not affected at all the patients who used them.
- The use of advanced techniques for metallic biomaterials characterization represents a step forward in understanding the effect of the human body onto these materials unlike the typical characterization of metallic implants impact on the human body.
- Optical and electronic microscopy are the main ways to evaluate the structural integrity of a metallic biomaterial since they highlight the fatigue and stress micro-fractures. Moreover, these techniques can also be used for assessing the structure of new materials.
- By coupling X-ray emitted and ICP-MS techniques, the chemical composition of unknown samples can be thoroughly assessed and this method can be extended for known materials in order to assess metal loss of a given metallic alloy.
- Analysis methods presented within the current paper are complementary to the ones already used before and can give a better idea of the material transformation during usage.

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