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# Determination of metallic traces in kidneys, livers, lungs and spleens of rats with metallic implants after a long implantation time

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**Abstract** Metallic transfer from implants does not stop at surrounding tissues, and metallic elements may be transferred by proteins to become lodged in organs far from the implant. This work presents an in vivo study of metallic implant corrosion to measure metallic element accumulation in organs located far from the implant, such as kidneys, livers, lungs and spleens. The studied metallic implant materials were CoCr alloy, Ti, and the experimental alloy MA956 coated with α-alumina. The implants were inserted in the hind legs of Wistar rats. Analysis for Co, Cr, Ti and Al metallic traces was performed after a long exposure time of 12 months by Inductively Coupled Plasma (ICP) with Mass Spectrometry (MS). According to the results, the highest Cr and Ti concentrations were detected in spleens. Co is mainly found in kidneys, since this element is eliminated via urine. Cr and Ti traces increased significantly in rat organs after the long implantation time. The organs of rats implanted with the α-alumina coated experimental MA956 did not present any variation in Al content after 12 months, which means there was no degradation of the alumina layer surface.

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## Introduction

A common source of metal contamination in the body comes from metallic implants such as plate components (cranial or maxillofacial), knee or hip joint prostheses, dental implants, clips for ligation, heart valves, etc. The metallic materials most frequently used in the human body are iron-based alloys of 316L stainless steel type, cobalt-based alloys, titanium-based alloys such as Ti-6Al4V, and commercially pure titanium (≥98.9%). 316L stainless steel may corrode inside the body in certain circumstances in highly stressed and oxygen-depleted regions such as the contacts under screws of the fracture plate. Stainless steels are vulnerable to pitting and crevice corrosion around screws. CoCr alloy and titanium-based alloys are widely used in orthopaedic applications due to their excellent corrosion resistance resulting from the formation of very stable, continuous, highly adherent and protective oxide films on the metallic surface. Most failures of these materials are due to fatigue and wear combined with the presence of a corrosive medium, such as a saline environment, giving rise to fatigue-corrosion and fretting-corrosion or wear-corrosion phenomena.

The corrosion of these metallic orthopaedic implant materials gives rise to metallic release that is initially localised in the first few microns of the surrounding bone. However, metallic contamination does not stop

there, and metallic elements may be transferred by albumin and transferrin proteins to become lodged in organs like kidneys, livers, lungs and spleens [1, 2]. For instance, Ti ions have been observed in the livers and spleens of patients with TiAlV hip prostheses [2]. Other investigations indicate that Al(III) released into the synovial fluid from aluminium alloy-containing hip or knee prosthetic joints, such as Ti6Al4V, may allow the potentially deleterious delivery of this metal ion to sites where it can exert toxicological effects [3]. Transferrin is the main means of transport for Al(III) in blood plasma. This complex may participate in the onset of neurological conditions, since the passage of this metal ion across the blood-brain barrier appears to be facilitated by the brain cell transferrin receptor [4, 5]. Strong evidence exists for the toxicity of Al in renal failure patients and it seems clear that Al availability, and therefore toxicity, depends on the physicochemical form of the element [6]. Others metals such as Cr, Ni, and Cd have been found to be potentially carcinogenic, and recent studies of stomach carcinoma support the hypothesis that Ni and Cr are carcinogenic agents [7]. Some authors highlight the contamination of organs like the liver with Mn and the lymphatic glands with Cr [8].

The corrosion of these metallic implants has already been studied by many authors [9, 10] in simulated physiological fluids [11, 12], in different aggressive conditions (pH, aeration) [13] and in cell cultures [14, 15]. Many measurements of metallic traces (such as Cr, Co, Ti) have been carried out in the blood and serum of patients with knee or hip prostheses [16–19] and the metallic contamination of periprosthetic tissues has also been studied [20]. However, the evaluation of metallic traces in organs far from the prostheses has been little studied [21, 22], only in cases such as post-mortem patients [23]. This paper assesses the accumulation in organs such as kidneys, livers, lungs and spleens of metallic traces released from different metallic implants after a long implantation time of twelve months in Wistar rats. The metallic implants considered in this study have been Co–Cr alloy, commercially pure titanium, and the experimental iron-based MA956 alloy. MA956 is interesting as a biomaterial because of its ability to generate in situ an adherent α-alumina layer of 3–5 μm thickness after heat treatment at 1,100 °C [24, 25]. This layer provides an excellent barrier against aggressive environments such as human body fluids and increases both corrosion resistance and biocompatibility. The MA956 alloy has been widely studied by our group for biomedical applications from the point of view of mechanical properties [26, 27], corrosion resistance [28, 29, 30] and in vitro biocompatibility [31]. Inductively Coupled Plasma (ICP) with Mass Spectrometry (MS) has been used to identify the different types of metallic traces lodged in rat organs [32].

## Materials and methods

The chemical composition of the studied metallic implants, as-received, was as follows: Co–Cr alloy 35Co–35Ni–20Cr–10Mo (wt.%); cp Titanium 99.7 (wt.%); and a base-Fe superalloy (MA956) Fe–20Cr–4.5Al–0.5Ti–0.5$Y_2O_3$ (wt.%).

All the metallic materials were machined to form cylinders of 6 mm in length and 1 mm in diameter, as shown in Fig. 1. The cylindrical specimens were rounded at one end in order to eliminate sharp edges and facilitate their insertion in the laboratory animals. The specimens were abraded using consecutively less abrasive SiC papers and polished with an alumina suspension of 1 μm. The specimens were ultrasonically cleaned in alcohol and then sterilised by UV radiation.

After this the MA956 alloy was preoxidised at 1,100 °C for 50 h to generate an α-alumina layer of a thickness of 4 μm on its surface.

The cylindrical implants were inserted by identifying the femoral condyles and drilling an orifice of 1.1 mm in diameter through the intercondylar notch to the central marrow channel. The animals used in the experiments were female albina Wistar rats of three months of age and weighing approximately 200 g. The MA956 implants were inserted in the right hind legs of all the rats, while the CoCr implants were inserted in the left hind legs of half of the rats and the cp Ti implants in the left hind legs of the other half (see Scheme 1). As an example, Fig. 2 presents SEM micrographs of a cross section of the preoxidised MA956 superalloy implanted in the right hind leg. The alumina coating is strongly adhered to both the metallic substrate and the bone. Each material was tested, in three different rats. The animals were slaughtered after 12 months and various organs were retrieved: kidneys, livers, lungs and spleens. A total of 36 organs were analysed. The experimental detail is summarised in Scheme 1.

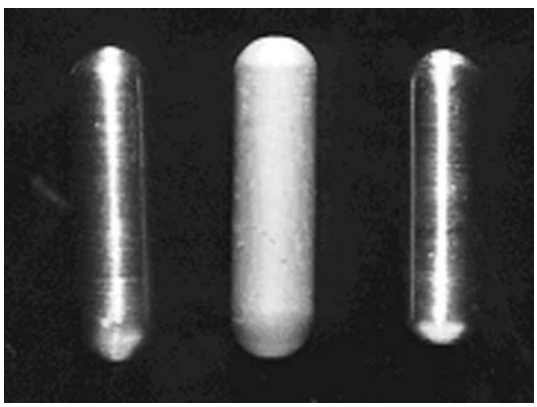


**Fig. 1** Metallic materials inserted as implants in the hind legs of Wistar rats (X10)

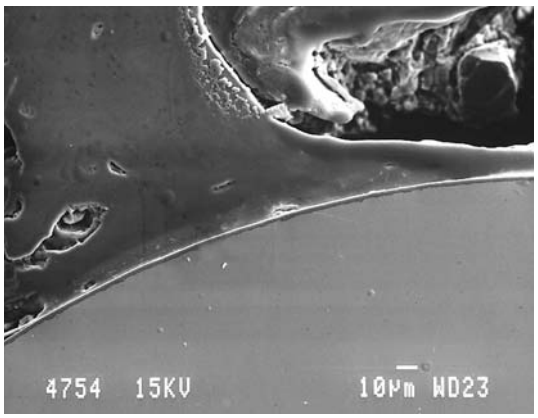
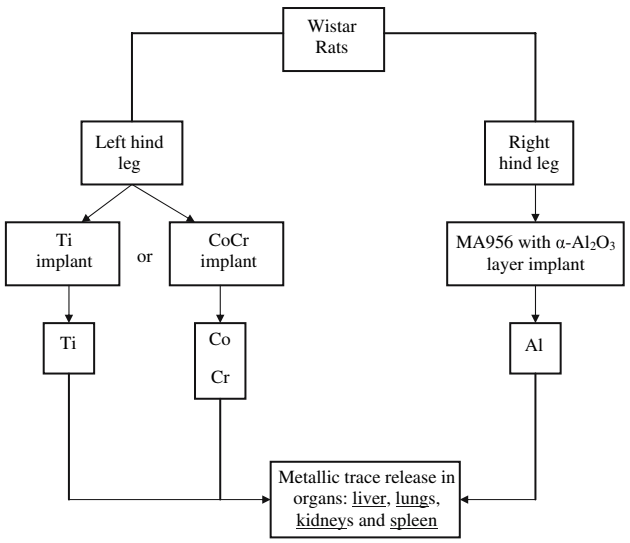



**Fig. 2** SEM micrograph of a cross section of MA956 alloy coated with α-alumina implanted in the hind leg of a rat




**Scheme 1** Experimental design of in vivo test

Control biological tissues: kidneys, livers, lungs and spleens were obtained from control rats not subjected to any kind of implant. NIST Standard Reference Materials SRM 1577-b Bovine Liver and SRM 8418 Bovine Muscle Powder were also used for reference purposes.

Prior to the analysis of metallic traces by ICP-Mass Spectroscopy (ICP-MS), the organs were dried in a oven at 110 °C for 48 h. The dried samples were then mixed manually and powdered in an agate mortar. About 500 mg of each powdered sample and 4 ml of nitric acid were placed in a Milestone HPV-80 vessel, which was then set on a carousel and subjected to the microwave heating programme indicated in Table 1. The digest was evaporated nearly to dryness on a hot-plate, then spiked with an internal standard of 1.0 µg of Sc and diluted to 25 ml with 0.1% $HNO_3$. A reagent blank was treated in the same way as the samples and taken through the entire digestion process. Each solution was analysed with an ELAN 500 ICP mass spectrometer (Perkin-Elmer Sciex, Norwalk, Ct, USA) and a FIAS-200 flow injection system (Perkin-Elmer, Überlingen, Germany).

Analyses were performed for the following metallic elements: Co, Cr, Ti and Al. The detection limit calculated on the basis of the standard deviation of ten successive measurements of the blank solution, using the $3\sigma$ criterion, was 0.2 ppm. Details of the experimental method for aluminium can be found in [32].

Data obtained in the determination of metallic traces in the various organs was subjected to statistical analysis using Statgraphics. This programme calculates average and extreme values and the variance for each study group, yielding significant results. The dispersion between averages and the quotient between the dispersion of averages and the experimental dispersion was also calculated.

## Results

Figures 3 and 4 show the Co and Cr trace values (ppm), respectively, detected in the kidneys, livers, lungs and spleens of rats with Co–Cr implants after 12 months of implantation. Co metallic traces appear principally in kidneys, since this element is easily excreted via urine, while Cr appears principally in spleens and also in very similar concentrations in kidneys and lungs.

Figure 5 shows the Ti analysed in the kidneys, livers, lungs and spleens of rats inserted with cp Ti implants for 12 months. Ti accumulates at surprisingly high levels in spleens (around 20 ppm) and to a lesser extent in livers and lungs. Figure 6 shows the same information, in this case for Al, detected in the organs of all the rats with alumina-coated MA956 implants. Al appears mainly in spleens and also in kidneys and lungs. By observation of Figs. 4–6 it can be seen that the organs with the highest metallic trace contamination are, in general, the spleen followed by the lungs.

Figures 7 and 8 show the sum of Cr and Co traces accumulated in each analysed organ and divided by the number of organs of rats with Co–Cr and MA implants, respectively.

The abscissa axis has been labelled Ti-MA for rats with a Ti implant in their left hind legs and an MA956 implant in their right hind legs. The denomination CoCr-MA refers to rats with a CoCr implant in their left hind legs and a MA956 implant in their hind right legs. In Fig. 7 it can be seen that rats with CoCr-MA956 implants present a significant increase in Cr traces in their organs compared to rats with Ti-MA956 implants. Figure 8 shows that there is no significant variation in Co traces.

**Table 1** Microwave heating programme

| Parameter | 1 | 2 | 3 | 4 | 5 | 6 | 7 |
|---|---|---|---|---|---|---|---|
| Power (W) | 250 | 0 | 250 | 250 | 300 | 0 | 300 |
| Time (min) | 0.5 | 2 | 0.5 | 5 | 2 | 2 | 5 |

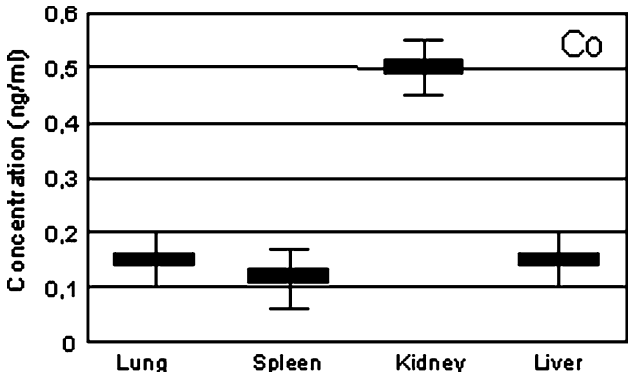



**Fig. 3** Distribution of Co metallic traces (ppm) detected in the kidneys, livers, lungs and spleens of rats with Co–Cr implants after 12 months of implantation

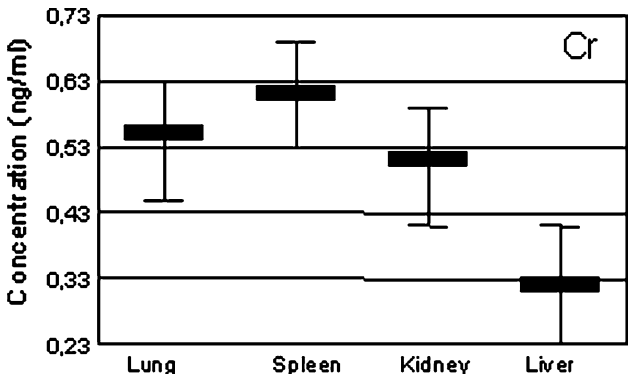



**Fig. 4** Distribution of Cr metallic traces (ppm) detected in the kidneys, livers, lungs and spleens of rats with Co–Cr implants after 12 months of implantation

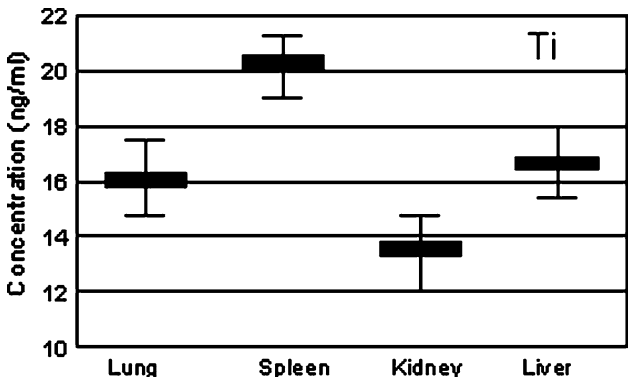



**Fig. 5** Distribution of Ti metallic traces (ppm) detected in the kidneys, livers, lungs and spleens of rats with Ti implants after 12 months of implantation

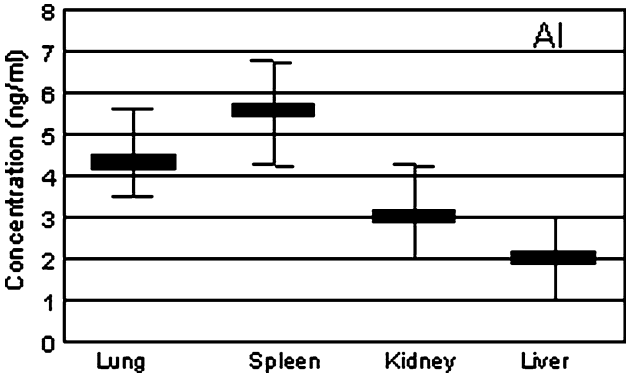



**Fig. 6** Distribution of Al metallic traces (ppm) detected in the kidneys, livers, lungs and spleens of rats with MA956 implants after 12 months of implantation

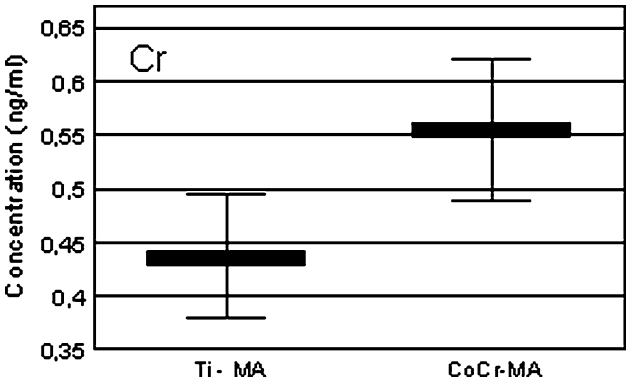



**Fig. 7** Analysis of Cr metallic traces of all the tested organs analysed by groups of rats (CoCr-MA)

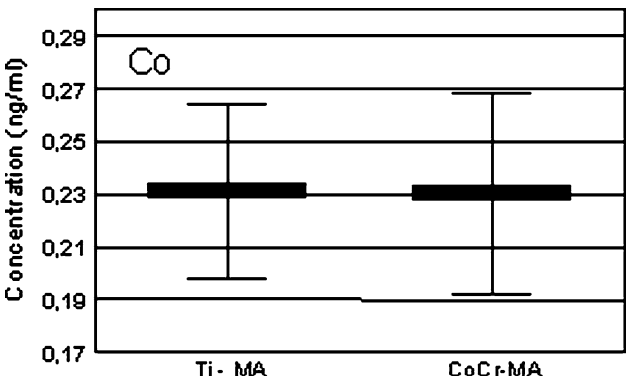



**Fig. 8** Analysis of Co metallic traces of all the tested organs analysed by groups of rats (CoCr-MA)

Figure 9 shows the sum of Ti traces accumulated in each analysed organ divided by the number of organs of rats with Ti and MA implants. In this case a significant increase can be seen in the organs of rats with Ti-MA956 implants compared to the organs of rats with CoCr-MA implants.

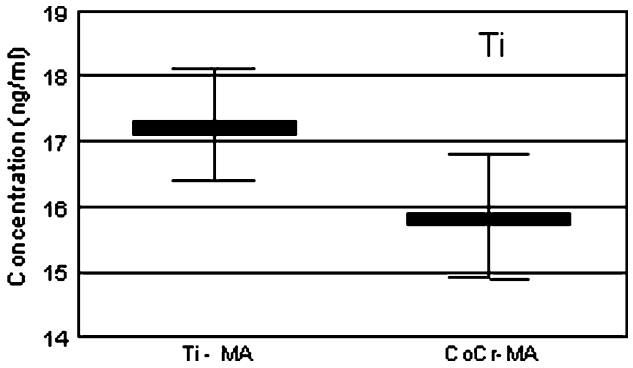



**Fig. 9** Analysis of Ti metallic traces of all the tested organs analysed by groups of rats (Ti-MA)

Figure 10 shows the sum of Al traces accumulated in each analysed organ and divided by the number of organs of rats with Co–Cr and MA implants and Ti and MA implants. The measurement of this element was compared with the control rats. There is no difference between Al levels in rats inserted with the MA956 implant and control rats.

## Discussion

When medical devices are inserted in the human body, the surface of the materials comes into contact with the living tissue. In the case of implanted metallic materials, corrosion of the implant surface leads over time to the in vivo release of metallic ions. The elements released from prostheses are stored by cells or are transferred to other organs to be eliminated. The wear corrosion process in joint replacement prostheses has been understood primarily in the context of the local effect of particle-induced periprosthetic, osteolysis and aseptic loosening [33–35]. The emigration of metallic traces from prostheses to far away organs has been less studied and is less understood. The

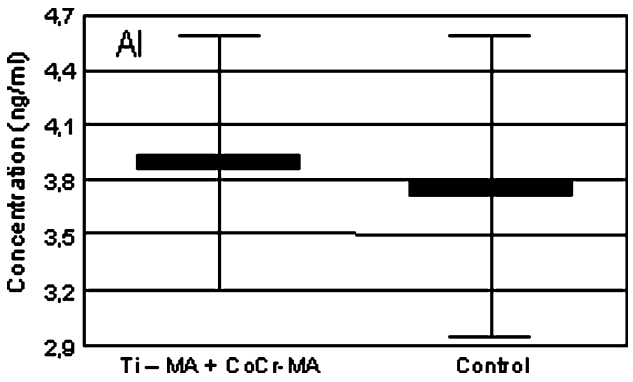



**Fig. 10** Analysis of Al metallic traces in the organs of all the tested rats (CoCr-MA and Ti-MA) compared to control rats

present work seeks to study the in vivo corrosion process of various metallic materials used as biomaterials, measuring in rat organs (kidneys, livers, lungs and spleens) the metallic traces from implants inserted in the animals' legs after a long implantation time of twelve months.

The presence of Cr and Ti in the organs of rats with Co–Cr and Ti implants and the insignificant presence of these elements in rats without this type of implants (compare Figs. 7 and 9) evidence the possibility of detecting the slow but continuous corrosion process and ion release from Ti and Co–Cr implants. However, considering that the kinetics of passive materials are very slow, it is necessary to wait 12 months (which can be extrapolated by a factor of 30× for humans) to be able to detect significant metallic traces in rat organs. Experimentation with rats using this type of implants (Co–Cr and Ti) inserted for 1, 3 and 6 months did not reveal any significant accumulation of metallic traces in organs.

With Co–Cr implants, the presence of Cr metallic traces (Fig. 7) and the absence of Co (Fig. 8) confirms that in in vivo tests the passive film is comprised mainly of a hydrated chromium oxyhydroxide. When this passive film comes into contact with body fluids the presence of chloride ions causes the breakdown and repassivation of the film, giving rise to a corrosion process. Such passivation and repassivation phenomena in the presence of chloride ions have previously been assessed by the authors with this type of CoCr implants in in vitro studies in a Hank's solution [36]. The particular chemical nature of solution-phase Cr(III) complexes arising from the potentially deleterious corrosion of chromium-containing alloy implants during in vivo wear episodes has been reported in an experimental model [19].

With Ti implants, the degradation of the passive layer, typically 4–6 nm in thickness and composed of amorphous or poorly crystallised and non-stoichiometric $TiO_2$ [37] film, shows a corrosion phenomenon over time. Ti appears to be accumulated as a metallic trace in rat organs (Fig. 9). In vitro Ti behaviour in Hank's solution is very different to the in vivo behaviour of this material [36, 38]. This difference in behaviour may be due to the fact that the passive film composition and properties can change over time, depending on the in vivo environment [39, 40]. This change has been observed by means of high resolution transmission electron microscopy, in which it has been seen that proteins are incorporated in the reconstructed surface oxide in addition to Ca, P and S. The surface oxide film is not always stable and its composition changes through the incorporation of ions and molecules in vivo [38]. Amino acids and proteins may be simple biochemical factors in ion release [38, 41, 42]. Research has shown that the Ti surface oxide is oxidised by more active oxygen species generated by macrophages adhering to the surface.

This mechanism is apparently one of the biochemical factors for the release of Ti ions. The macrophages around the material also generate active oxygen species which accelerate Ti dissolution [38]. Other studies show that the repassivation of Ti in vivo is slower and that more Ti ions are released into the body fluids [30]. In conclusion, Ti is not always stable in vivo and reacts with ions and molecules to reconstruct a new surface oxide film. An understanding of metallic ion toxicity requires a knowledge of the amount of ions released, clarification of their toxicity, and consideration of the probability of combination with biomolecules such as proteins and enzymes.

With regard to MA956 implants, Fig. 10 shows that there is no significant increase in Al in rats with MA956 implants compared to control rats without implants. MA956 has shown excellent results in in vitro tests in a wide range of environments, at pH 3 and in different oxygenation conditions, simulating inflammation and infection processes [43]. These in vitro tests have been corroborated in in vivo experiments, since Al is not obtained as a significant trace in rats with this type of implant. The anodic polarisation curves performed in in vitro experiments revealed a very low probability of breakdown of the alumina layer and, in the event of the breakdown of the alumina layer, the excellent repassivation ability of the alloy due to its high Cr content (20%) assures the good corrosion behaviour of the alloy [43]. The MA956 implant, with an alumina layer of a thickness about 250 times higher than the passive layer, shows the corrosion behaviour of a quasi perfect dielectric that prevents corrosion of the metallic substrate. Moreover the degradation of the α-alumina after an implantation time of 12 months is not significantly appreciable in the various rat organs.

## Conclusions

The analyses of Co, Cr, Ti and Al metallic traces lodged in the different organs by ICP-MS was measured with a detection limit calculated with a precision of 0.2 ppm. This analytical method has made it possible to detect that the slow but continuous corrosion process on the surfaces of the CoCr alloy, Ti implants and the MA956 coated with α-alumina is not an occurrence of merely local significance but that it affects the trace element status of the entire organism. After a long time period of 12 months, the in - vivo ion release process is evident from the metal traces detected in the organs of rats with CoCr alloy and Ti implants. Of all the analysed organs, the spleen is that which presents the greatest accumulation of Cr, Ti, and Al traces. Co is excreted by urine, and so the greatest accumulation of Co traces is found in kidneys. Finally, the best in vivo corrosion behaviour is shown by the MA956 coated with α-alumina, which shows the lowest metal contamination in the organs of rats implanted with this material.

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