

# Titanium levels in the organs and blood of rats with a titanium implant, in the absence of wear, as determined by double-focusing ICP-MS

Alejandro Sarmiento-González · Jorge Ruiz Encinar ·  
Juan M. Marchante-Gayón · Alfredo Sanz-Medel

Received: 24 July 2008 / Revised: 26 September 2008 / Accepted: 30 September 2008 / Published online: 24 October 2008  
© Springer-Verlag 2008

**Abstract** Titanium (Ti) has long been regarded as an inert and biocompatible metal, ideal for biomedical applications such as dental implants or joint replacements. However, concerns about the biocompatibility of Ti have lately arisen. Unfortunately, information on reliable Ti baseline physiological levels in blood and organ tissues is still pending and the real effects of physiological corrosion as opposed to wear processes of Ti or Ti alloys implants is controversial so far. In this work a previously developed and validated methodology, based on using double-focusing inductively coupled plasma mass spectrometry (DF-ICP-MS) has been used to establish Ti basal levels in blood and organs (heart, liver, spleen, kidneys, and lungs) of Wistar rats. These data were compared with the levels found in three Wistar rats implanted with a Ti wire embedded in their femur for 18 months, in order to assign possible Ti released purely due to non-wear physiological mechanisms. Results showed that Ti content in all the selected organ tissues and blood was higher than previously determined Ti basal levels, clearly showing both corrosion of the Ti implant and systemic Ti accumulation in target tissues. These results indicate that Ti metal corrosion occurs. This seems to be the only mechanism responsible in the long term for the observed passive dissolution of Ti of the implant in the absence of wear. A comparative study of the systemic distribution of the soluble and particulate Ti potentially released from Ti implants was also carried out by intra-

peritoneally injection of soluble  $\text{Ti}(\text{citrate})_3$  and insoluble  $\text{TiO}_2$  particles, respectively. Different systemic Ti storage was observed. Whereas soluble Ti was rapidly transported to all distal organs under study,  $\text{TiO}_2$  particles were only accumulated in lung tissue.

**Keywords** Titanium · Implants · DF-ICP-MS · Rats · Corrosion

## Introduction

The treatment of rheumatoid arthritis and osteoarthritis by total joint replacement is an important health-care concern because of their prevalence in our expanding population of elderly patients. At present, 0.16–0.20% per year of the worldwide population receives a total hip joint arthroplasty [1]. However, current metallic implant technology is limited and all metallic implants degrade to some extent over time, resulting in increased serum and urine metal concentrations and, eventually, in local and systemic metal storage [2–5]. As metal degrades, the resulting metallic implant debris may exist “in vivo” as particles (micrometer to nanometre in size), as colloidal and ionic forms (non-specifically or specifically bound by proteins), colloidal organometallic complexes, and inorganic metal salts or oxides [6]. All those metal physical forms pose some biological risk for the organism, leading to many potential adverse effects including cytotoxicity, genotoxicity, carcinogenicity, and metal hypersensitivity [7–10]. Furthermore, such metallic debris may initiate acute and chronic inflammatory responses, and even necrosis, in the tissues surrounding the prostheses, contributing to bone resorption, which seems to be the major factor in the aseptic loosening of implants [11].

A. Sarmiento-González · J. R. Encinar (✉) ·  
J. M. Marchante-Gayón · A. Sanz-Medel (✉)  
Department of Physical and Analytical Chemistry,  
Faculty of Chemistry, University of Oviedo,  
Julián Clavería 8,  
33006 Oviedo, Spain  
e-mail: ruizjorge@uniovi.es  
e-mail: asm@uniovi.es

Metals are released from prostheses by numerous mechanisms, including corrosion, wear and mechanically assisted electrochemical processes such as fretting corrosion, stress corrosion and, corrosion fatigue. Identification of the exact process responsible for elevated metal levels observed in clinical studies [5, 12] is not an easy task, because such different mechanisms often act simultaneously and their extent depends not only on the material used but also on environmental factors (pH, implant locations, etc.). In any case, it must be taken into account that although, in the future, it might, perhaps, be possible to minimize (or ultimately eliminate) wear, releases via passive physiological dissolution will be always present. Thus, assessment of purely electrochemical release mechanisms is needed today in order to measure possible metal release even without a component of wear.

Titanium (Ti) has long been regarded as an inert and biocompatible highly corrosion-resistant metal, because of the thin and stable protective oxide layer spontaneously formed on its surface. However, implanted Ti undergoes events such as bending, scratching, or, according to recent studies, corrosion [13] which may disrupt the passive surface oxide layer. In the process of removal and reformation of this passive layer, metal ions and/or particles can be released and locally stored or mobilized. Unfortunately, and contrary to popular belief, such released Ti may cause harmful effects in blood, fibrotic tissues, or osteogenic cells after travelling through the circulatory or lymphatic systems [14]. For the above reasons, many questions about the biocompatibility Ti implants have lately been raised and many studies initiated and carried out on this topic coming from very different scientific disciplines (e.g. medicine, chemistry, biochemistry, materials science, etc.). Many of those studies focused simply on identification of Ti particles in target organs by use of the electron microprobe [15, 16] but Campbell et al. [17] demonstrated that metal released from an implant can be present as nanoparticle debris or as organometallic complexes (bound to tissue proteins) which can be beyond the resolution of the electron microprobe. On the other hand, most of the studies have tried to quantify the Ti present in the blood or organ tissues of implanted patients using a wide range of atomic analytical techniques (ETAAS, ICP-OES, ICP-QMS, etc.). Unfortunately, most of these techniques lack the sensitivity and selectivity required for such studies. As an example, Jacobs et al. [18] recently reviewed his experience on Ti serum determination in more than 750 individuals using ETAAS and concluded that Ti basal levels should be below their Ti detection limit (DL; 2.1 ppb). Their values were lower than those provided by other authors using the same technique (23.9 ppb [19], 3 ppb [20]). This sensitivity problem could not be overcome by using quadrupole-based ICP-MS, because the isobaric and polyatomic interferences with every Ti isotope, especially important when analysing biological samples, are

very serious, making matters even worse than using ETAAS. Moreover, interference attenuation using collision/reaction cell technology has proved to be insufficient to quantify Ti at the basal levels in complex samples by ICP-QMS [21, 22]. An additional concern in accomplishing reliable quantification of ultratrace levels of Ti present in such complex samples is contamination derived from inappropriate materials or sample pretreatments [4]. Finally, in some cases, reported data seem not to be correctly processed [5, 23] under adequate chemical metrology protocols [24]. All these factors have led to great confusion and controversy about true Ti physiological or baseline levels in fluids and organs of non-implanted people and, therefore, to a lack of quantitative evidence of the amounts of Ti released from a Ti-based implant in the absence of wear (of course, much lower than that observed when mechanical wear takes place).

The objective of this work was to shed some light on this issue of reliable Ti baseline levels in rat tissues typically used for modelling Ti release and distribution. To do so, we have resorted to a double focusing inductively coupled plasma mass spectrometer (DF-ICP-MS), the analytical technique of choice for the determination of Ti in biological samples, because of the possibility of measuring at medium resolution (avoiding spectral polyatomic interferences such as  $^{31}\text{P}^{16}\text{O}^+$  on  $^{47}\text{Ti}^+$ ) with low DL for Ti. The use of this powerful technique in conjunction with ultraclean sample-preparation procedures could enable assessment of “in vivo” corrosion of Ti implants in the absence of wear. The rat organ Ti distribution patterns observed will be critically compared with those obtained after intraperitoneal injection of soluble Ti ( $\text{Ti}(\text{citrate})_3$ ) and non-soluble Ti ( $\text{TiO}_2$  particles) in order to investigate the differential transport and distribution in the organism of both types of Ti species.

## Experimental

### Reagents and materials

Ultrapure deionized water ( $\geq 18.2 \text{ M}\Omega \text{ cm}$ ) was obtained from an Advance A10 Milli-Q system (Millipore, Bedford, MA, USA). Suprapur hydrogen peroxide 30% and analytical-reagent-grade nitric acid 65%, additionally purified by sub-boiling distillation, both from Merck (Darmstadt, Germany), were used for the microwave digestion of blood and tissue samples. Suprapur sodium chloride (99.99%) and  $1,000 \text{ mg L}^{-1}$  Ti and Ga ICP standards were also obtained from Merck. Non-commercially available solid natural titanium citrate was synthesized from  $\text{TiCl}_4$  (Fluka, Buchs, Switzerland) and citric acid monohydrate (Merck) following the procedure published by Deng et al. [25].

Ti wire (purity 99.99%) was chosen as the implant material. It was purchased from Sigma-Aldrich (Steinheim,

Germany). On reception the implant geometry corresponded to a cylindrical wire of 1.0 mm outer diameter. Sphere-like  $\text{TiO}_2$  particles, purchased from Fluka, were approximately 1  $\mu\text{m}$  in size.

Two different biological reference materials were used as quality controls in this study: Seronorm Trace Elements Urine Blank (ref. 201205, LOT NO 2525) and Seronorm Trace Elements Whole Blood (Level 1, LOT MR 4206); both were from Seronorm (Nycomed, Oslo, Norway).

For collection of blood samples, plastic collection tubes conditioned with lithium heparin were purchased from Vacuette (Greiner bio-one, Madrid, Spain).

Clean procedures were used in sampling and sample pretreatments. Metal and glassware were avoided and all plasticware was soaked in an acid bath of sub-boiling  $\text{HNO}_3$  10% (v/v) for at least 24 h, rinsed several times with water ( $\geq 18.2 \text{ M}\Omega \text{ cm}$ ), and then dried prior to use in a clean fume hood equipped with a laminar flow system.

#### Animals enrolled in the study

Treatment of the animals in this study followed ethical guidelines established by the University of Oviedo. Approval of all animal procedures was obtained from the local ethics committee. Sixteen male Wistar rats were used throughout the work and were divided into two different studies. In the first study seven four-month-old Wistar rats weighing approximately 200 g were enrolled. Three received a Ti wire implant for 18 months whereas the other four animals were used as control group (did not receive any implant).

A second related study was carried out with nine four-month-old rats divided into three different groups, each comprising three animals. One group was intraperitoneally injected with a suspension of titanium oxide ( $\text{TiO}_2$ ) particles in 0.9% (w/w) NaCl (group A); another group was intraperitoneally injected with a solution of the previously synthesized  $\text{Ti}(\text{citrate})_3$  in 0.9% (w/w) NaCl (group B) and, finally the control group intraperitoneally injected with 0.9% (w/w) NaCl (control group). None of the animals died or showed alterations in body weight, behaviour, or general health during the experiments.

#### Procedures

##### *Ti wire implantation*

Before implantation, and following the American Society for Testing and Materials (ASTM) standards for the preparation of metallic surgical implants, cylindrical Ti wire was soaked in 20% (v/v)  $\text{HNO}_3$  for 20 min, washed with water ( $\geq 18.2 \text{ M}\Omega \text{ cm}$ ), and, finally, sterilized by autoclaving. The animals were anaesthetized with isoflurane at 5% vaporized in  $\text{O}_2$  flowing at  $2 \text{ mL min}^{-1}$  prior to surgical

implantation. Following anaesthesia, an incision was made in the right hind leg under aseptic conditions. Afterwards, the Ti wire was inserted by identifying the femoral condyles and drilling with a Dremel bit (outer diameter 1.1 mm) an orifice through the intercondylar notch to the central marrow channel. The cylindrical shape ends were rounded in order to eliminate sharp edges. Sample migration was avoided by closing the fascia with a non-resorbable suture point. Figure 1 shows a radiograph recorded nine months after implantation. Note that radiographs of every implanted rat were recorded every three months to check the status of the prostheses.

##### *Synthesis of the non-commercially available $\text{Ti}(\text{citrate})_3$*

In order to study possible mobilization of a soluble  $\text{Ti}(\text{IV})$  species biocompatible with the body fluids, we carried out the synthesis of the non-commercially available  $\text{Ti}(\text{citrate})_3$  ( $(\text{NH}_4)[\text{Ti}(\text{H}_2\text{Cit})_3] \cdot 3\text{H}_2\text{O}$ ) following the procedure published by Deng et al. [25]. Briefly, 1.9 g (10 mmol)  $\text{Ti}(\text{IV})$  tetrachloride and 6.3 g (30 mmol) citric acid monohydrate were mixed and the pH of the solution was adjusted to 2.0 by slow addition of dilute ammonia solution. The solution



**Fig. 1** Radiograph of Ti wire embedded in the femur of a rat, taken 9 months after surgical implantation

was continuously stirred and heated gently until a white microcrystalline material started to form. The precipitate was then collected and recrystallized from hot water. The Ti(IV) final reaction yield was 80%, as determined by ICP-MS using standard additions. The synthesized compound chemical characterized by NMR, IR, and C, H, and N elemental analysis; the data obtained (not shown) agreed well with those published by Deng [25].

#### *Intraperitoneal injection of the TiO<sub>2</sub> particles suspension and of the Ti(citrate)<sub>3</sub> solution*

Both the suspension of TiO<sub>2</sub> particles and Ti(citrate)<sub>3</sub> solution were vortex mixed and intraperitoneally administered in a single dose of approximately  $2.0 \times 10^4$  ng Ti in 1 mL 0.9% (w/w) NaCl saline solution. Controls were given intraperitoneal injections of equivalent volumes of the saline solution (0.9% (w/w) NaCl) to compensate for the effect of the vehicle.

#### *Blood samples collection*

Extraction of blood from implanted rats was performed every 6 months in order to evaluate Ti level changes in blood over time. Samples were withdrawn using a stainless steel needle surrounded by an inert plastic cannula. After insertion into the heart, the needle was removed and blood was sampled through the plastic cannula. Blood was drawn into standard plastic syringes previously cleaned. The first 1 mL of blood was used to rinse the system and then discarded. The second 2 mL were transferred to blood-collection tubes conditioned with lithium heparin and stored at  $\leq 18$  °C until analysis. Prior to the animals being euthanized, isoflurane anaesthesia at 5% vaporized in O<sub>2</sub> was used; the animals were then sacrificed by exsanguination via cardiac puncture to allow blood collection.

#### *Organ tissue samples collection*

Different organs (liver, kidneys, heart, spleen, and lungs) from every implanted rat were immediately isolated, lyophilized for 72 h, and individually stored at  $\leq 18$  °C until analysis. Organ tissue samples from the intraperitoneally injected Wistar rats were pooled after excision and homogenised prior to the 72 h lyophilization step. Afterwards, pooled samples were stored at  $\leq 18$  °C until analysis.

#### *Blood samples pretreatment*

Whole blood (1 mL) was accurately weighed, using a Precisa Instruments (Dietikon, Switzerland) XB220A analytical balance, into microwave digestion vessels. Diluted sub-boiling HNO<sub>3</sub> (1:3 v/v, 8 mL) and 1 mL H<sub>2</sub>O<sub>2</sub> were added to the

digestion vessels and digested by following the microwave program:

1. 95 °C for 3 min;
2. 160 °C for 10 min; and
3. 185 °C for 18 min.

The digests were transferred to pre-cleaned low-density polyethylene (LDPE) containers, spiked with Ga as internal standard to obtain 10 ppb concentration in the final solution, and diluted to 15 mL in water ( $\geq 18.2$  MΩ cm).

#### *Organ tissue samples pretreatment*

Previously lyophilized and homogenized organ tissues were also microwave digested. For this purpose, approximately 250 mg organ tissue sample were accurately weighed and subjected to the same sample pre-treatment as blood samples. It is worth stressing that microwave digestion vessels were thoroughly cleaned before all the sample digestions making use of the same reagents and microwave program previously mentioned but in the absence of the sample. Moreover, at least one digestion blank was prepared for every 10-samples carousel digested.

#### *Calibration and quality control*

Six-point external calibration curves from 0 to 5 µg L<sup>-1</sup> Ti were constructed every five working hours. Regression coefficients obtained were always excellent ( $r^2$  ranged from 0.9996 to 1.0000). Every 12 samples, a blank and a Ti ICP standard of the calibration curve (2.5 µg L<sup>-1</sup> Ti) were measured to check for memory effects and correct for likely signal drift. In addition, two selected Ti-containing biological reference materials were also measured every 12 samples as a quality control (if each reference material result was not within the confidence interval of the reference value the whole group analysis was repeated).

#### *Apparatus*

Element-specific detection of Ti was performed using an Element 2 (Thermo Fisher Scientific, Waltham, MA, USA) double-focusing inductively coupled plasma mass spectrometer (DF-ICP-MS) working at medium resolution ( $R_S=4000$ ) in order to avoid the polyatomic spectral interferences with Ti determination, especially in human blood sample analysis. The ICP-MS was equipped with an ASX-510 autosampler for 4×60 samples (CETAC Technologies, Omaha, USA). Operating conditions used can be found elsewhere [2]. In this work only <sup>47</sup>Ti, <sup>49</sup>Ti, and <sup>71</sup>Ga were measured.

Organ tissue samples were lyophilized in a Heto Lyolab 3000 freeze-drier model from Heto-Holten (Allerød, Denmark). After lyophilization, dried tissues were ground and homoge-



nized in an agate mortar. For whole blood and organ tissues digestion, a model Ethos 1 microwave oven (Milestone, Socisole, Italy) equipped with ten middle pressure PTFE vessels and a temperature sensor was used.

## Results and discussion

### Quality control

In order to check the quality of the quantitative results obtained during the whole analysis run (20 h), two biological Ti-containing reference materials were analyzed after every set of 14 samples (including a blank and a calibration standard):

1. whole blood (reference value:  $2.3 \mu\text{g L}^{-1}$ , confidence interval:  $1.3\text{--}3.3 \mu\text{g L}^{-1}$ ); and
2. urine (reference value:  $4.9 \mu\text{g L}^{-1}$ , confidence interval:  $3.41\text{--}6.41 \mu\text{g L}^{-1}$ ).

Results obtained for both reference materials during the analysis period are shown in Fig. 2. As can be clearly seen, every quantitative result lay within the confidence interval for each reference material. Moreover, the data-to-data reproducibility obtained during the whole working period was very good ( $2.24 \pm 0.30$  and  $4.78 \pm 0.62 \mu\text{g Ti L}^{-1}$ , for the whole blood and urine, respectively,  $n=18$ ), demonstrating the robustness of the method.

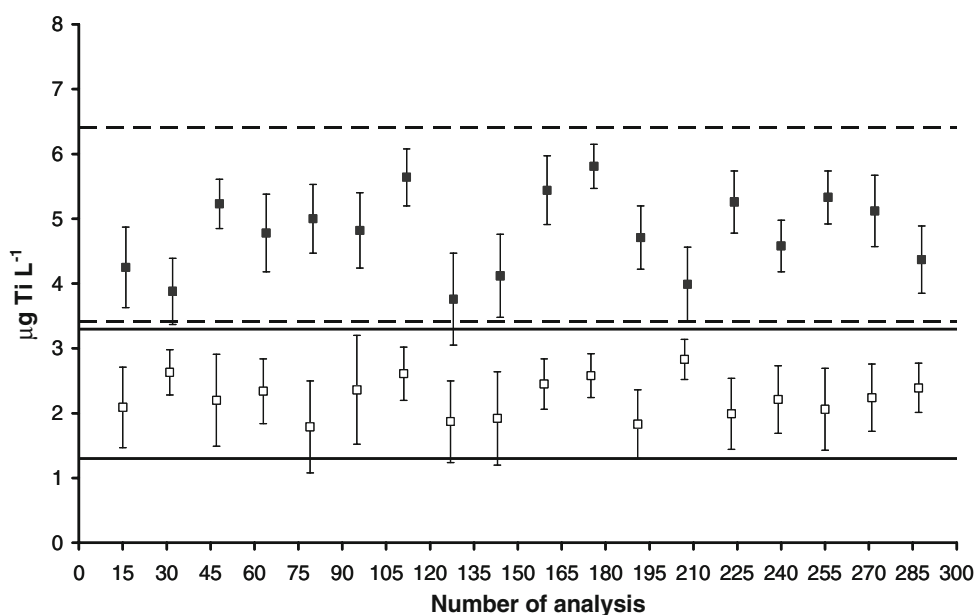
### Implanted animals

Quantitative analysis of Ti following the previously detailed procedures was carried out on liver, kidneys, spleen, lung, and heart tissue from the three implanted Wistar rats. First, Ti levels

were determined in a pool of each of the five target organs of the control group (non-implanted) in order to establish Ti baseline levels. It is worth stressing that determination of reliable Ti basal levels could be achieved for each organ because of the extremely high sensitivity provided by the DF-ICP-MS used. In fact, after acidic microwave digestion and dilution with water ( $\geq 18.2 \text{ M}\Omega \text{ cm}$ ) of the different organs of the control group, the corresponding Ti levels in solution to be quantified in the range  $0.2$  to  $0.6 \mu\text{g L}^{-1}$  Ti, which were well above the DL of our approach ( $0.066 \mu\text{g L}^{-1}$  Ti). This DL was calculated by following IUPAC guidelines (3 times the standard deviation of 10 consecutive measurements of the blank method solution). Such values not only back the reliability of the Ti basal levels given in this work, they also indicate the unreliability of other much higher reported values (Table 1) and the need to use DF-ICP-MS in these studies.

Data obtained for the Ti content of organs from the three implanted animals are individually shown in Fig. 3, together with controls (white columns). As can be observed, elevated Ti levels, as compared with our previously established Ti basal levels, could be found in all the organs under study for the three implanted animals. These results demonstrate unequivocally that corrosion in the absence of wear had taken place, because Ti was released and then mobilized to all of the five organs selected for this study. For further clarification and comparison of the results, Ti average content in organ tissues of the control group and implanted animals are shown in Table 2. As can be observed, the spleen, a part of the immune system, showed the highest Ti content of all the distal tissues (closely followed by the lungs). Much lower Ti concentrations, but always well above baseline levels, were observed in kidney and heart tissues. In contrast, the liver results obtained were

**Fig. 2** Reproducibility obtained for determination of Ti in the reference materials used as quality controls along the whole analysis run (20 h). *Black and white squares* correspond to Ti determination in the urine and whole-blood reference materials, respectively. *Dashed and solid lines* correspond to the confidence interval for the urine and whole-blood reference materials, respectively



**Table 1** Illustrative results reported in the literature for the Ti content of spleen and lung tissues of animals with a Ti implant in the absence of wear

Spleen (ng g <sup>-1</sup> )	Lung (ng g <sup>-1</sup> )	Detection Limit (μg L <sup>-1</sup> )	Ref.
90±28	24.3±1.0	23.9	[19]
1370±28	895±19	Not given	[27]
20000±1000	16000±1000	200	[26]

Detection limits obtained are also provided when available

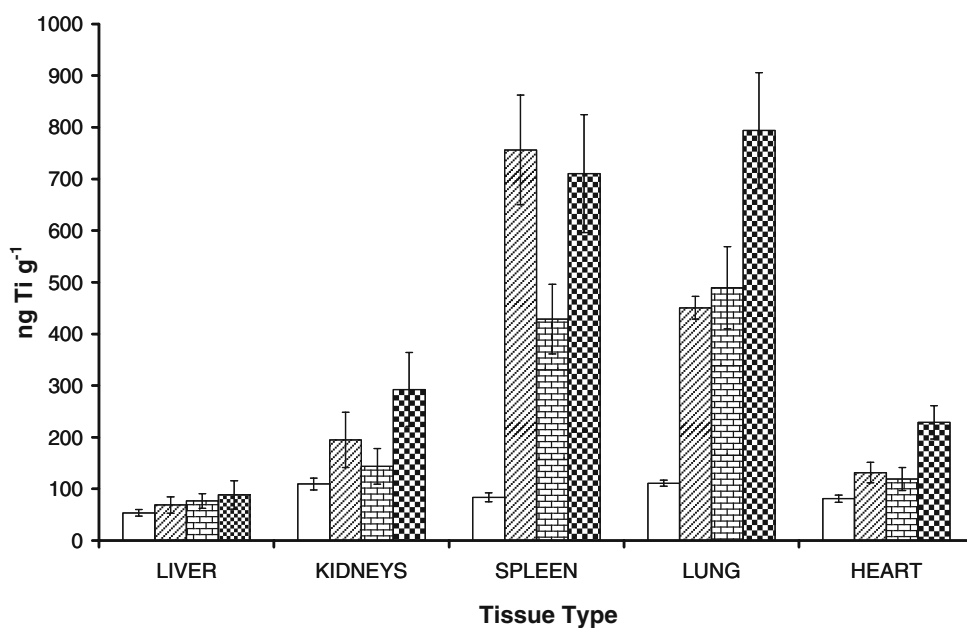
hardly increased in comparison with those found in the control group (despite its detoxification function).

Of course, the previously stressed limitations of other techniques and published articles on completely reliable Ti baseline levels and of robust quantification of low levels of Ti in biological samples will influence the corrosion results controversy. As an example, Table 1 collects some of the results published so far [19, 26, 27] for spleen and lung tissues of animals with a Ti implant in the absence of wear (these two tissues were selected here for comparison purposes because they showed the highest Ti concentrations). Both, data and conclusions drawn in those articles [19, 26, 27] were quite different. Despite the high Ti concentrations reported in lung and spleen tissue, only Rubio et al. [26] claimed Ti concentration enhancements over the reported controls. However, those authors did not include true controls in their study because all the animals enrolled in that work were implanted with different kinds of material, hampering comparisons. Moreover, taking into account that Ti concentrations in blood greater than 8 ppb could be possible only in the presence of an important component of wear, aseptic loosening of the implant, or the

presence of multiple Ti-containing implants [18], it is most unlikely that the reported elevated values could be ascribed just to corrosion mechanisms. A conventional ICP-MS without any kind of polyatomic interference suppression was used. Therefore, Ti determination most likely suffered from serious polyatomic interferences. This is confirmed by the fact that reported Ti DL were 200 ppb [26] (extremely high compared with those previously obtained by us [2]). A similar reason could explain the high Ti results reported by Rodríguez et al. [27], also using a conventional quadrupole ICP-MS. On the other hand, Bianco et al. [19] used ETAAS for the purpose and Ti concentrations observed in lung and spleen tissues of the implanted rats were not statistically different from the values observed by us in the controls. It is worth stressing, however, that Ti concentration observed for the implanted and control rats were most of the time below the DL (23.9 μg L<sup>-1</sup> Ti) of that technique, so quantitative conclusions are debatable. In contrast, and as previously mentioned, our low detection and quantification limits allowed Ti basal levels to be established clearly and, therefore, detection of even slight Ti increases (e.g. due to corrosion processes).

Potential Ti implant corrosion, needed before the observed systemic storage of Ti in the different target organs under study takes place, was then investigated through periodic analysis of the blood of the implanted rats. Ti blood concentrations obtained after different periods of implantation are shown in Fig. 4. As can be observed, after an implantation time of 6 or 12 months, Ti concentration levels in blood did not differ significantly from those of the control group. Only for the blood collected at the end of the study, 18 months after implantation, was the mean blood Ti content

**Fig. 3** Ti found (ng g<sup>-1</sup> dry tissue) in the different organ tissues of the three implanted and four non-implanted (controls) rats. *White columns* correspond to the Ti content obtained from analysis of each one of the pooled organs of the four animals comprising the control group. The three *filled columns* show the Ti content determined for each of the three implanted rats (uncertainty corresponds to one standard deviation of three independently prepared sample aliquots)



**Table 2** Ti content (ng g<sup>-1</sup> dry tissue) found in the organs of controls and of implanted rats 18 months after implantation

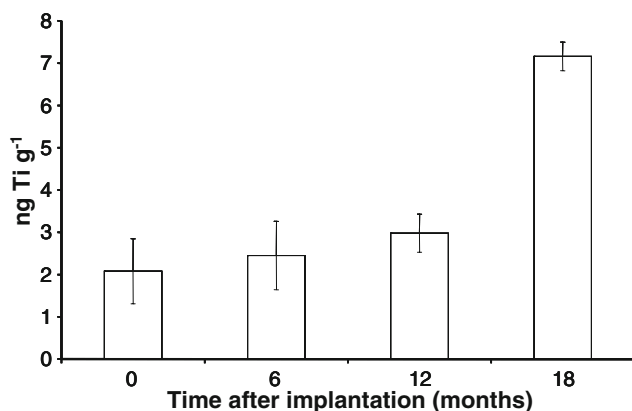
Tissue type	Controls (n=4)	Implanted (n=3)
Liver	53.9±6.2	78.1±9.8
Kidneys	110±11	210±76
Spleen	83.8±8.7	632±177
Lungs	111±6	578±189
Heart	81.4±7.0	160±60

Control values correspond to the analysis in triplicate of the pooled organs of four animals with no implants. Implanted values correspond to averages of the individual data obtained for each of the three implanted animals (uncertainty corresponds to one standard deviation)

clearly higher than observed Ti basal levels. This could indicate that partial disruption of the Ti oxide film surface [13] could occur after 12 or more months of implantation. Whole blood and organ tissue (Fig. 4 and Table 2) results obtained support that corrosion, the only mechanism likely in this study, could be responsible for passive Ti dissolution of the implant and its further systemic storage. It is worth stressing that, to the best of our knowledge, this is the first time that both Ti blood and Ti organ contents elevation have been reported “in vivo” for a Ti implant in the absence of wear. These findings are tied to the use of DF-ICP-MS, an analytical technique selective and sensitive enough to allow reliable Ti determination in the very low ppb range even in complex biological matrices.

#### Experiments with intraperitoneally injected animals

In order to shed further light on the “in vivo” Ti corrosion results (from implants in the absence of wear), we injected intraperitoneally two different types of Ti species into Wistar rats. Then, Ti distribution was measured and compared for both types of Ti exposure. As a model of particulate “non-

**Fig. 4** Ti concentration found in three periodic extractions of blood of the implanted rats. Values correspond to the average of the individual data obtained for each of the three implanted animals (uncertainty corresponds to one standard deviation)

water soluble Ti species”, a suspension of TiO<sub>2</sub> particles was selected. Conversely, our model for “water soluble Ti(IV) species” at physiological pH (7.4), was titanium citrate. It is interesting to note that to the best of our knowledge, this is the first report using a water-soluble, biologically compatible Ti species to investigate Ti mobilization. As described above, three different groups each comprising three rats were intraperitoneally injected with:

1. a suspension of TiO<sub>2</sub> particles in a 0.9% (w/w) NaCl saline solution (group A);
2. Ti(citrate)<sub>3</sub> completely dissolved in 0.9% (w/w) NaCl saline solution (group B); and
3. a reference group injected only with the 0.9% (w/w) NaCl saline solution (to be used as controls).

The high sensitivity provided by the DF-ICP-MS allowed us to use comparatively low Ti(citrate)<sub>3</sub> and TiO<sub>2</sub> doses. It is worth stressing in this connection that some previous works used less sensitive Ti detection techniques [15, 28]. Thus, much higher doses had to be used, likely disturbing rat metabolism and therefore, interpretation of results was much more difficult. In fact, TiO<sub>2</sub> particles and Ti(citrate)<sub>3</sub> were injected here in one single dose of 2.0×10<sup>4</sup> ng Ti, far away from Ti doses reported in literature, ranging from 1.6×10<sup>6</sup> to 1.6×10<sup>8</sup> ng Ti [15, 28].

Most common sizes of metal TiO<sub>2</sub> particles found in retrieval studies after joint replacement are in the range 1–10 μm [29]. In this study, we selected low-diameter 1 μm TiO<sub>2</sub> particles, because it is known that toxicity increases as their size decreases [30]. One week after intraperitoneal injection, all the nine animals were sacrificed and quantitative Ti determinations of the five studied organ tissues were carried out. A one-week experiment duration was selected in order to allow observations of the likely different organ Ti distributions for injected soluble and for particulate Ti. Table 3 shows the results obtained for the five organ tissues of the three groups (A, B, and controls). Interestingly, quite different behaviour was obtained for Ti (citrate)<sub>3</sub> and TiO<sub>2</sub> particles. Ti concentrations in all the

**Table 3** Ti content of the corresponding organs (ng g<sup>-1</sup> dry tissue) and blood (ng g<sup>-1</sup>) of intraperitoneally injected rats

Tissue type	Controls	Ti(citrate) <sub>3</sub>	TiO <sub>2</sub>
Liver	63.0±5.0	375±16	63.3±4.7
Kidneys	140±10	2433±63	132±9
Spleen	104±9	2527±52	134±13
Lungs	129±10	681±29	918±64
Heart	86.2±4.2	276±30	160±14
Blood	2.36±0.48	6.10±0.59	5.88±0.43

Data correspond to the analysis in triplicate of the pooled organs of three animals (uncertainty corresponds to one standard deviation)

organ tissues of the animals injected with  $\text{Ti}(\text{citrate})_3$  (group B) were much higher than those obtained in the control group (rats simply injected with the 0.9% (w/w) NaCl saline solution). Conversely, in the animals injected with  $\text{TiO}_2$  particles (group A) only the lung tissue showed significantly higher Ti levels than those obtained for the control group. Also, Ti levels found in lungs were six times higher than those found in heart. This seems to indicate that lungs can constitute a likely final sink of the small  $\text{TiO}_2$  particles entering the circulatory system. Olmedo et al. [31] observed that  $\text{TiO}_2$  particles were transported in blood by phagocytic monocytes and deposited mainly in the lungs, but also in the liver and spleen (heart tissue was not analyzed) six months after intraperitoneal injection. Their comparatively high concentrations of  $\text{TiO}_2$  doses assayed (from  $10^2$  to  $10^4$  times higher than those used in our study) could explain those findings (e.g. likely saturation of the organism detoxification capabilities might occur under such experimental conditions [31]).

For Ti from  $\text{Ti}(\text{citrate})_3$  injections, the spleen again led the list of the organs with the highest Ti content, followed by kidneys (Ti values quite close to those found in the spleen are apparent in Table 3). Finally, lung, liver, and heart analysis also provided Ti concentrations well above those obtained in control rats. Interestingly, Ti distribution patterns in organs of the implanted rats (Table 2) resembled much more that obtained for the soluble Ti species injected. In both cases, the spleen seems to be the principal target organ for Ti. Also, Ti levels found in the lungs were significantly higher than the values observed for controls. This result points to the possibility that a significant part of the Ti released from the Ti implant seems to be soluble Ti. It is interesting to note also that only the water-soluble Ti species,  $\text{Ti}(\text{citrate})_3$ , was found at a significant level in the kidneys and liver (both having detoxifying function) which seems to indicate that soluble Ti is mobilized to a much greater extent than non-soluble Ti.

Ti levels found in blood extracted when the animals for the three studied groups (groups A, B, and controls) were euthanized, are also shown in Table 3. As can be observed, a nearly three-fold increase Ti concentration was found, for both groups A and B, in comparison with the control group. Interestingly, those Ti levels are close to those observed for the implanted rats 18 months after implantation (Fig. 4).

## Conclusions

The extremely low DLs provided by DF-ICP-MS ( $0.066 \mu\text{g L}^{-1}$ ) enabled reliable determination of Ti in all the organ tissues and blood analyzed, even at basal levels and independently of the chemical form of the Ti. To the best of our knowledge, we believe that this is the first time that reliable

basal Ti levels in blood and organ tissues of Wistar rats can be claimed. These basal values could serve as a reference in further studies and in other scientific disciplines. We consider that the relevance of this study is clearly interdisciplinary and might contribute to overcoming some of the present problems of quality and transparency of reported Ti quantitative results in biological tissues and fluids. The reliable determination of such Ti basal levels enables quantitation of minor elevations in mean Ti blood content in the 18-months-implanted animals. The results obtained demonstrate also that corrosion from Ti implants occurs and could be responsible of the observed significant systemic storage of Ti, mostly in the spleen and lungs.

Different systemic storage was observed for soluble Ti ( $\text{Ti}(\text{citrate})_3$ ) and insoluble Ti ( $\text{TiO}_2$  particles). Whereas Ti from  $\text{Ti}(\text{citrate})_3$  seems to be rapidly transported to all the organs under study,  $\text{TiO}_2$  particles were preferentially accumulated in lung tissue. Interestingly, the Ti distribution pattern observed for soluble Ti when intraperitoneally injected as  $\text{Ti}(\text{citrate})_3$  was similar to that of Ti released from the Ti implant. This fact suggests that most of the Ti released from the implant seems to be soluble Ti which should then be transported to different organ tissues, most likely bound to transferrin [32].

Finally, we expect that data presented here could shed light on reported controversial Ti basal levels in blood and organs and on possible corrosion of a Ti implant in the absence of wear. These are the two most important aspects of understanding the real inertness and possible side effects of biomedical applications of Ti metal and its alloys.

**Acknowledgements** Financial support from the MEC (Madrid, Spain, CTQ2006-02309/BQU) and Applera Hispania (FUO-EM-023-05) is gratefully acknowledged. J.R.E. acknowledges the MEC and the European Social Fund for a Ramon y Cajal contract. The authors gratefully acknowledge Agustín Brea and Teresa Sánchez from the Biotery of the University of Oviedo for their help and kind suggestions.

## References

1. Sargeant A, Goswami T (2007) *Mater Design* 28:155–171
2. Sarmiento González A, Marchante Gayón JM, Tejerina Lobo JM, Paz Jiménez J, Sanz Medel A (2008) *Anal Bioanal Chem* 391:2583–2589
3. Savarino L, Granchi D, Ciapetti G, Cenni E, Nardi Pantoli A, Rotini R, Veronesi CA (2002) *J Biomed Mater Res* 63:467–474
4. Zeiner M, Zenz P, Lintner F, Schuster E, Schwägerl W, Steffan I (2007) *Microchem J* 85:145–148
5. Jacobs JJ, Skipor AK, Patterson LM, Hallab NJ, Paprosky WG, Black J, Galante JO (1998) *J Bone Joint Surg [Am]* 80(A):1447–1458
6. McDonald SJ, McCalden RW, Chess DG, Bourne RB, Rorabeck CH, Cleland D (2003) *Clin Rel Res* 406:282–296
7. Bagchi D, Stohs S, Downs B, Bagchi M, Preuss H (2002) *Toxicology* 180:5–22
8. Liu K, Husler J, Ye J, Leonard S, Cutler D, Chen F (2001) *Mol Cell Biochem* 222(12):221–229



9. Quievryn G, Peterson E, Messer J, Zhitkovich A (2003) *Biochemistry* 42(4):1062–1070
10. Hallab NJ, Merritt K, Jacobs JJ (2001) *J Bone Joint Surg [Am]* 83A:428–436
11. Clohisy JC, Calvert G, Tull F, McDonald D, Maloney WJ (2004) *Clin Orthop Relat Res* 429:188–192
12. Iavicoli I, Falcone G, Alessandrelli M, Cresti R, De Santis V, Salvatori S, Alimonti A, Carelli G (2006) *J Trace Elem Med Biol* 20:25–31
13. Hanawa T (2004) *Mater Sci Eng C* 24:745–752
14. Wang ML, Tuli R, Manner PA, Sharkey PF, Hall DJ, Tuan RS (2003) *Orthop Res* 4:697–707
15. Olmedo DG, Tasat DR, Guglielmotti MB, Cabrini RL (2005) *J Biomed Mater Res* 73A:142–149
16. Olmedo DG, Tasat DR, Guglielmotti MB, Cabrini RL (2008) *J Mater Sci Mater Med* 19:3049–3056
17. Campbell PM, Urban RM, Catelas I, Skipor AK, Schmalzreid TP (2003) *J Bone Joint Surg [Am]* 85:2218–2222
18. Jacobs JJ, Skipor AK, Campbell A, Hallab NJ, Urban RM, Amstutz CJ (2004) *Arthroplasty* 19(8):59–65
19. Bianco PD, Ducheyne P, Cuckler JM (1997) *J Mater Sci Mater Med* 8:525–529
20. Bianco PD, Ducheyne P, Cuckler J (1996) *Biomaterials* 17:1937–1942
21. Sarmiento González A, Marchante Gayón JM, Tejerina Lobo JM, Paz Jiménez J, Sanz Medel A (2005) *Anal Bioanal Chem* 382:1001–1009
22. McGarry S, Morgan SJ, Grosskreuz RM, Williams AE, Smith WR (2008) *J Trauma* 64:430–433
23. Savarino L, Greo M, Cenni E, Cavasinni L, Rotini R, Baldini N, Giunti A (2006) *J Bone Joint Surg Br* 88B:472–476
24. Ellison SLR, King B, Rösslein M, Salit M, Williams A (Lisbon, 2003) *Traceability in Chemical Measurement. EURACHEM/CITAC Guide*
25. Deng F, Zhou ZH, Wan HL (2004) *Inorg Chem* 43:6266–6273
26. Rubio JC, García-Alonso MC, Alonso C, Alobera MA, Clemente C, Munuera L, Escudero ML (2008) *J Mater Sci Mater Med* 19:369–375
27. Rodríguez D, Gil FJ, Planell JA, Jorge E, Álvarez L, García R, Larrea M, Zapata A (1999) *J Mater Sci Mater Med* 10:847–851
28. Olmedo D, Guglielmotti MB, Cabrini RL (2002) *J Mater Sci Mater Med* 13:793–796
29. Dorr LD, Milton KR, Wan Z, Markovich GD, Bloebaum R (1996) *Clin Orthop* 333:108–117
30. O'Connor DT, Choi MG, Kwon SY, Paul Sung KL (2004) *J Orthop Res* 22:229–236
31. Olmedo DG, Tasat DR, Guglielmotti MB, Cabrini RL (2003) *J Mater Sci Mater Med* 14:1099–1103
32. Tinoco AD, Valentine AM (2005) *J Am Chem Soc* 127 (32):11218–11219