World Journal of Advanced Research and Reviews
eISSN: 2581-9615 CODEN (USA): WJARAI
Cross Ref DOI: 10.30574/wjarr
Journal homepage: https://wjarr.com/

(REVIEW ARTICLE)

Check for updates

Metals and metal ions release from metallic implants in the animal body models: A review of experimental investigations

Magdalena Richter [1] and Henryk Matusiewicz [2,*]

[1] *Department of Orthopedics and Traumatology, Poznan University of Medical Sciences, Poznań, Poland.*
[2] *Faculty of Chemical Technology, Poznan University of Technology, Poznań, Poland.*

World Journal of Advanced Research and Reviews, 2022, 16(01), 653–676

Publication history: Received on 06 September 2022; revised on 16 October 2022; accepted on 19 October 2022

Article DOI: https://doi.org/10.30574/wjarr.2022.16.1.1024

Abstract

All non-noble metals and alloys will release metallic species into the body. This raises the issue of amount and fate, i.e. transport and storage, of these metal dissolution products. No metal or alloy is completely inert *in vivo*. 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. The use of metallic biomaterials in the medical implant devices has become increasingly prevalent over the past few decades. Metals and metal ions release from metallic materials; titanium and titanium and its alloys, nickel-titanium alloys, cobalt-chromium alloys, and magnesium-based alloys implanted into the human body is becoming a major cause of concern. The development of reliable experimental models for the clinical use of biomaterials and in predicting implant success or failure is becoming increasingly important in attaining adequate health and safety conditions. Animal models provide important biomaterial knowledge that eventually leads to the development of more effective clinical treatments of diseases in both humans and animals; therefore, acting as a bridge between *In vitro* studies and *in vivo* clinical trials. The most commonly used laboratory animals have turned out to be rats, mice, and rabbits, probably because they are cheaper and easy to handle. In addition, cattle, primates, sheep, swine and guinea pigs are used by researchers in a progressively decreasing order. The present review reported the results of *In vitro* and *in vivo* experimental investigations on the release of metallic traces in the biological fluids, tissues and different inner organ tissues resulting from the implantation of the small metallic implants in the animals.

Keywords: Metallic implants; Animal model; Metallic traces; Metal ion release; Biological fluids and organ tissues; Analytical methodology

1. Introduction

All non-noble metals and alloys from medical metallic implants are release metallic species into the body. This raises the issue of amount and fate, i.e. transport and local and systemic metal storage, of these metal dissolution products [1]. Metals from medical implants are released into surrounding tissues by various mechanisms, including corrosion, wear, and mechanically accelerated electrochemical processes such as stress corrosion, corrosion fatigue and fretting corrosion. This metal release has been associated with clinical implant failure, osteolysis, cutaneous allergic reactions, and remote site accumulation [2]. It is now recognized that biomaterial capacity to perform a specific function in patients cannot be evaluated only *In vitro* or *in vivo*, and that also *In vitro* testing, unfortunately, does not exclude *in vivo* testing [3]. *In vitro* methods provide necessary data to supplement those found *in vivo*, usually accepted, firstly, as a first choice method for testing material safety and, secondly, as a method for testing separate phenomena to gain a deeper insight into the *in vivo* mechanisms of actions that are fundamental in determining failure or success of implantation surgery. In addition, *in vivo* methods can reduce the number of animals used for *in vivo* biocompatibility evaluations and

* Corresponding author: Henryk Matusiewicz
Faculty of Chemical Technology, Poznan University of Technology, Poznań, Poland.

since the implantation of toxic biomaterials in animals can be avoided through preliminary *In vitro* tests, there is a reduction in animal suffering. Biomedical implants should be subjected to both *In vitro* and *in vivo* studies for their application. Although *In vitro* studies give an insight of the behavior of the material under the given conditions, it should not be regarded as the final conclusion for recommending a material as an implant, whereas *in vivo* tests examine the actual performance of the implant within the animal models [4]. Animal models have been widely used in biomedical research and have been crucial for acquiring basic science and clinical knowledge pertaining to medical science. Animal models provide important biomaterial knowledge that eventually leads to the development of more effective clinical treatments of diseases in both humans and animals, therefore, acting as a bridge between *In vitro* studies and clinical trials [5].

The present review reported the results of *In vitro* and *in vivo* experimental investigations on the release of metallic traces in the biological fluids, tissues and different inner organs from the implantation of the small metallic medical implants in the animals. Furthermore a compilation of the results (metals and metal ion studies) that have so far been published in the literature is presented. Other aspects leading to the release of *in vivo* and *In vitro* metal ions and potential related adverse physiological effects, including toxicity, carcinogenicity, genotoxicity and metal allergy, are beyond the scope of this contribution and will not be discussed here. It is not within the scope of this review to evaluate possible toxicological effects produced by the intake of trace metals, nor to estimate tolerable animal body intakes.

2. *In vitro* and *in vivo* tests for the biological evaluation of titanium implants

Titanium and Ti-6Al-4V alloy have been recognized as metals with an excellent biocompatibility. Titanium and its alloys, like the majority of metallic implant materials, release passive metal dissolution products. This raises the issues of amount and fate, i.e. transport, storage, and/or excretion of these metal dissolution products. However, literature information concerning the titanium ion levels in tissues associated with the use of Ti-6Al-4V implants is limited. Comparison between studies is difficult, due to the use of different animal models and the lack of a common procedure for implantation used in each study.

2.1. Titanium ions release from titanium implant materials

Metal implants, or more often alloyed metal implants, are commonly used structural elements (have are predominated in orthopedic surgery), which support the functions of the human body, in particular the skeletal system. In implantology, austenitic steels, metal alloys (Co-Cr-Mo; Co-Cr; Co-Ni; Cr-Ni), tantalum, zirconium, tungsten, titanium, titanium alloys, magnesium alloys are used. Among them, one of the most frequently used biomaterials are conventional titanium (cp-Ti) and titanium-based alloys (Ti-6Al-4V, Ti-Al-Nb, Ni-Ti) and can be considered as the most corrosion-resistant of the alloys described. Although, titanium shows a better resistance to corrosion, the release of small amounts of corrosion products (titanium, aluminum and vanadium ions from the Ti-6Al-4V implant alloy) is still possible, even through a stable oxide film of titanium dioxide (TiO_2), and thus this layer is not inert [6]. However, prolonged contact of the body with an implant causes a number of unwanted effects, which result in structural changes in the implant itself, reducing the lifetime and systemic toxicity.

In this section below, information on the metal ion release from titanium implant materials into various tissues, fluids and inner organs of animals is presented, according to the empirical data. Ferguson and coworkers [7,8] were the first to document locally elevated titanium levels in the presence of a titanium implant. After a 4-6 month implantation period of titanium plugs in rabbits, they reported approximately a 20-fold increase in titanium quantities in the muscle adjacent to the implant as compared to controls. For the majority of rabbits, there was no difference in titanium levels between controls and rabbits with the implants. However, some of the experimental rabbits showed elevated titanium levels in spleen and lung tissue. Inconsistencies prevent the use of these data in making a sound conclusion on the presence or absence of systemic transport of titanium. Although there is some debate over "normal" titanium levels in tissues, there is agreement that the levels measured by Ferguson at al. [7,8] are several orders of magnitude too high. It can be speculated that sample contamination may have been responsible for this discrepancy. In addition, the number of implants per site was not consistent. As a result, the surface area of titanium varied from rabbit to rabbit. Although no one has studied the release of aluminum and vanadium from Ti-6Al-4V alloys, Ferguson et al. [9] demonstrated in animals a marked increase in aluminum concentration surrounding implants of aluminum 2024-T3 as well as elevated aluminum levels in spleen and liver. The exact mechanism for titanium release is not clear, but early reports by Ferguson at el. [7-9] showed that ionization of all metal occurs to some extent and titanium may be released in relatively large concentrations into adjacent tissues. Aluminum alloys implanted in rabbit muscle in a study by Laing et al. [10] showed little tissue reaction in spite of heavy contamination (500 to over 1,000 $\mu g\ g^{-1}$) of the surrounding muscle. This decreased only slightly with anodization, which significantly reduced macroscopic corrosion.

Titanium and other elements released from titanium implants are sometimes detected in tissues near the implants and organs [11-16] when implanted *in vivo* , despite its high corrosion resistance, mostly due to repetitive destruction of the film by wear and fretting [11]. Some of them are also detected in the absence of wear [12-16]. In 1984, Woodman et al. [11] attempted to quantify the amount of titanium released from a Ti-6Al-4V prosthetic segmental replacement in the long bones of baboons. There was no statistical difference between experimental and control animals in serum titanium concentrations. A six-fold increase was measured in the urine of the experimental group (implantation time 36-92 months) compared to controls. Titanium is detected in tissue around titanium implant. There were inconsistencies of titanium levels in local muscle. Lung, spleen, and regional lymph node samples of baboons with implants had consistent increase in titanium levels in comparison to controls. No kinetic data were presented for serum and urine. A kinetic analysis was given for the spleen and lungs. However, interpreting these data is difficult due to several experimental problems. The implant design was complex. It had several parts made of different alloys of titanium. In addition, stainless steel screws were used. Thus, the likely presence of fretting and galvanic corrosion and wear could have been factors that increased the scatter of the data. Time affects the diffusion of metals from implants to bone or other organ tissues such as liver, kidney, and muscles. Seki [12] used rabbits as an animal model. Author report the titanium and aluminum concentration in subcutaneous tissue around various implanted metallic plates made of pure titanium, titanium alloy, and titanium nitride. The amount of titanium eluted from cp-Ti, Ti-6Al-4V and TiN, increased with time, although only slightly in all cases. However, the Al concentrations in the tissues surrounding the Ti-6Al-4V alloy are 276, 287, 164, 232 μg g^{-1} dry weight after 2, 4, 8 and 12 weeks, respectively, and they are considerably higher than the Ti concentrations. Histological examination of the reactions of bone tissue to all titanium implants, showed the formation of new bone between the metallic screw and bone tissue from the 4th week, and it gained a close and dense contact with normal bone at the 12th week after implantation. Bianco et al. [13] document titanium levels in tissues local to commercially pure titanium (cp-Ti) implant in the absence of wear; compare these values to control tissues; and determine the relative contribution of the local accumulation to total release. Titanium fiber felts were implanted into the tibia of rabbits for periods up to 1 year; the samples were analyzed for titanium content. Compared to controls, titanium levels in the bone near the implant were elevated at 1-, 4-, and 12-month postoperative time points. The 12-month time point had higher periprosthetic bone titanium levels than both the 1- and the 4-month implant groups. The data support the hypothesis that metal species released from titanium implants in the absence of wear have a limited solubility. In another paper, the authors [14] report the titanium concentration in serum and urine of rabbits in the absence of wear. Titanium fiber felts were implanted into the tibia of rabbits. At selected time points up to 1 year postoperatively, titanium serum and urine concentrations were measured and compared with controls. The data for the implant group show that titanium levels in serum and urine do not increase in comparison to controls up to one year after implantation. The results presented here indicate that titanium released in the form and quantity from passive dissolution of the oxide is not transported in significant concentrations *via* serum proteins and is not measurable excreted in the urine. In the next paper, Bianco et al. [15] also used rabbits as an animal model. Authors investigated titanium levels in alleged target tissues in rabbits, both with and without a titanium implant functioning in the absence of wear. Porous Ti-6Al-4V fiber felts were implanted into the tibia of the rabbits. At various time points, lung, spleen, and muscle samples were analyzed for titanium concentration. The data for the implant groups show that titanium levels in these tissues do not increase in comparison with controls up to 1 year after implantation. The binding of proteins to passive dissolution products implies that there is a potential for systemic transport and accumulation. However, the data for the *in vivo* study did not find any increases in titanium serum or urine concentrations [14] or titanium content in any of the remote tissues (present study) that could be uniquely associated with the titanium implant. Therefore, if any passive dissolution products-to-protein binding mechanism occurs, there is only minimal transport of the passive dissolution products. Ektessabi et al. [16] reported the results of application of micro ion beam PIXE spectroscopy to detect release of titanium from titanium and titanium alloy implants inserted in the tibiae of rabbits for three months. It was found that titanium ions could be detected in the surrounding tissues, as a gradient from the implant surface and in higher amounts in the bone tissue as compared with the soft tissues.

Lugowski et al. [17] determined the amount of Al, Cr, Co, Mo, Ni, Ti, and V in organs (brain, liver, lung, kidney, and spleen tissues) in 2 years rabbit from an *in vivo* implantation experiment. Because there were no true controls, i.e. rabbits with no implants, the rabbits with non-titanium containing implants were considered as controls. The authors used rabbits with hydroxyapatite, Co-Cr-Mo alloy or alumina implants as controls. No differences between either the rabbit with a dense Ti-6Al-4V disc or the rabbits with a porous Ti-6Al-4V disc and the control rabbits were found in any of the analyzed tissues.

The effect of implantation time and implant nitriding on titanium ion concentration in several tissues of rats carrying Ti-6Al-4V implants was studied [18]. Spleen, muscle, kidney, lung, brain and bone samples were retrieved from these rats as well as from the control group. The tissue samples examined did not show evidence of systemic accumulation of titanium ions due to implantation of a Ti-6Al-4V alloy sample. The highest of titanium ion levels were measured in spleen samples. The values obtained are consistent with those of literature [11, 15, 17], considering the different animal

models and exposed surface/weight ratio. The high levels of titanium measured on spleen samples (30% over the other measurements) could be due to the metabolic function of spleen, as evidence exists that spleen is an accumulation organ for metals. Although the authors have developed careful trace-element procedure [19], this report leaves the understanding of systemic distribution patterns of titanium largely untouched. The use of biased controls and a small sample size eliminates any statistical comparison. Furthermore, the implantation procedures, such as the implantation site, are not detailed. An *In vitro* study using rabbits by Mu et al. [20], found the main sources of metal ion release to be handling during surgery, titanium implantation *in vivo* , and wear and fretting during the time the metal (pure titanium) was in the rabbit body for 48 weeks. The damage to the oxide layer can occur from mechanically assisted corrosion or handling during surgery leading to corrosion in the body. Major causes of titanium release were surgical handling in implantation and wear and/or fretting during experimental-term for 48 weeks. Titanium was detected in soft tissues and tibia near implants in muscles, 9.6% that of osteotomies, indicating that titanium is released from implants in the absence of wear and fretting due to certain biochemical factors. A recent study showed that metal ions are released from titanium with active oxygen species generated by macrophages [21]. Active oxygen species are one of the causes of titanium release in the absence of wear and fretting. Frisken et al. [22] reported a methodology for the determination of titanium at $\mu g\ g^{-1}$ level of dissemination of titanium from threaded screw type implants following placement of single implants in sheep mandibles. Twelve sheep were implanted with a single self-tapping implant for time intervals of one, four and eight to 12 weeks. Four un-operated sheep served as controls. Regional lymph nodes, lungs, spleen and livers were dissected and subsequently analyzed. Results associated with successful implants showed no statistically significant different levels of titanium in any organ compared to controls, although some minor elevations in titanium levels within the lungs and regional lymph nodes were noted. Debris from a single implant insertion is at such a low level that it is unlikely to pose a health problem. However, this study suggested that implant failure may result in considerably more titanium release which can track through the regional lymph nodes.

Rubio et al. [23] presented 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 material was Ti. The implant was inserted in the hind legs of Wistar rats. Analysis for Ti metallic traces was performed after a long exposure time of 12 months. According to the results, the highest Ti concentration was detected in spleens. Rubio et al. [23] 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. Sarmiento-González et al. [24] has been established 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 inner organ 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 *in vivo* of Ti of the implant in the absence of wear and its further systemic storage, mostly in the spleen and lungs.

To compare metal concentrations in tibia tissues with various metallic implants, Ti-6Al-4V and V-free Ti-15Zr-4Nb-4Ta alloys were implanted into the rat tibia for up to 48 weeks. Minute quantities of Ti, Al and V in the tibia tissues with the Ti-6Al-4V implant were found. The Ti concentration in the tibia tissues with the Ti-15Zr-4Nb-4Ta implant was lower than that in the tibia tissues with the Ti-6Al-4V implant. The Zr, Nb and Ta concentrations were also very low. The Ti-15Zr-4Nb-4Ta alloy with its low metal release *in vivo* is considered advantageous for long-term implants [25,26].

The concentrations of Ti in the biological (fluid, organ tissue) samples of animals following ion release from titanium and titanium alloy implants are presented in Table 1 and Table 2.

Table 1 Ti concentration in body fluids and inner organ tissues of animals following ion release from titanium and titanium alloy implants

Animal	Implant material	Implant specification	Implantation data		Body fluid/organ tissue analyzed	Metal ion(s) measured	Concentration (µg L-1)		Analytical technique	Research results	Refs.
			No. animals	Implantation time			Metal levels	Control levels			
Baboons	Ti-6Al-4V alloy	Titanium fiber sleeve	45	3 w to 92 m *in vivo*	Serum	Ti	2.71	2.75	GF-AAS	Serum chemistry and hematological analysis did not indicate a statistically significant difference in any of the parameters measured when comparing the group with implants to control group.	[11]
						V	0.27	0.26			
						Al	427	264			
					Urine	Ti	13.5	2.0			
						V	1.07	1.21			
						Al	603	631			
White rabbits	Titanium fibre felt	Discs	21	2 w and 1,2,4,6,9,12 m *in vivo*	Serum	Ti	5.15	4.14	GF-AAS	Titanium levels in serum and urine do not increase in comparison to controls up to one year after implantation.	[14]
					Urine	Ti	11.68	8.04			
Wistar rats	Ti-6Al-4V alloy	Cylindrical plates	21	30 and 120 d	Lung	Ti	812, 801	740	ICP-MS	The tissue samples did not show evidence of systemic accumulation of Ti ions due to implantation of Ti-6Al-4V implant.	[18]
					Muscle	Ti	921, 1035	1060			
					Kidney	Ti	887, 868	882			
					Spleen	Ti	1370,1381	1659			
					Liver	Ti	108, 805	850			
		Plates, screws	8	48 w	Osteotamies	Ti	9.45	n/a	GF-AAS		[20]

White rabbits	cp-Titanium			*in vivo*	Musck	Ti	0.98			Major causes of Ti release from bone plate-screw sets implanted into the legs of rabbits were surgical handling in implantation and wear and/or fretting.	
Wistar rats	cp-Titanium	Cylinders	3	12 m *in vivo*	Kidney	Ti	13	n/a	ICP-MS	The *in vivo* ion release process is evident from the Ti traces detected in the organs of rats with titanium implant.	[23]
					Liver	Ti	17				
					Lung	Ti	16				
					Spleen	Ti	20				
Wistar rats	Titanium	Wire	16	18 m	Liver	Ti	78.1	53.9	ICP-MS	The most of the Ti released from the titanium implant seems to be soluble Ti which should then be transported to different organ tissues, most likely bound to transferrin.	[24]
					Kidney	Ti	210	110			
					Spleen	Ti	632	83.8			
					Lung	Ti	578	111			
					Heart	Ti	160	81			

Analytical techniques: ICP-MS, inductively coupled plasma mass spectrometry; GF-AAS, graphite furnace atomic absorption spectrometry; Abbreviations: n/a-data not available; d-days; w-weeks; m-months

Table 2 Ti concentration in inner organ tissues of animals following ion release from titanium and titanium alloy implants

Animal	Implant material	Implant specification	Implantation data		Organ tissue analysed	Metal ion(s) measured	Concentration (µg g-1)		Analytical technique	Research results	Refs.
			No. animals	Implantation time			Metal levels	Control levels			
Baboons	Ti-6Al-4V alloy	Ti fiber sleeve	45	3 w to 92 m in vivo	Lung	Ti	958	137	GF-AAS	There were inconsistencies of titanium levels in local muscle. Lung, spleen, and regional lymph node samples of baboons with implants had consistent increases in titanium levels in comparison to control. It was found systemic accumulation of titanium in the spleen and lung.	[11]
						V	2.89	2.94			
						Al	1297	281			
					Kidney	Ti	1.90	13.25			
						V	1.81	2.01			
						Al	101	108			
					Spleen	Ti	90.63	20.15			
						V	1.78	1.66			
						Al	179.31	214.40			
					Liver	Ti	36.3	15.64			
						V	2.61	2.56			
						Al	139.74	166.80			
					Muscle	Ti	17.20	14.21			
						V	3.11	2.97			
						Al	133.71	139.43			
					Regional lymph nodes	Ti	524.33	98.71			
						V	1.64	1.76			
						Al	573.07	74.13			

Rabbits	cp-Titanium Ti-6Al-4V TiN	Plates, screws	64	2,4,8,12 w in vivo	Subcutane-ous	Ti	11.5 µg g-1		ICP-OES	All experimental materials exhibited excellent resistance to corrosion. Titanium nitride is useful as a biomaterial the same as cp-Ti and Ti-6Al-4V alloy.	[12]
						Ti	14.4				
						Al	231.6				
						Ti	8.1				
White rabbits	Titanium-based implant	Titanium fiber felt	40	1,4,12 m in vivo	Local muscle	Ti	32 ng g-1	28	GF-AAS	Compared to controls, Ti levels in the bone near the implant were elevated at postoperative time points. Titanium levels in muscle tissue near the felt also were occurring.	[13]
					Local bone	Ti	750	410			
Rabbits	Ti fibre felt	Discs	35	1,4,12 m	Lung	Ti	24.8	25.9	GF-AAS	The data for the implant groups show that Ti levels in these tissues do not increase in comparison with controls up to one year after implantation.	[15]
					Spleen	Ti	90.1	77.0			
					Muscle	Ti	< 25.7	-			
Sheep	cp-Titanium	Screws	12	1,4,8,12 m	Node	Ti	300	170	GF-AAS	This study showed no significant release of particle of titanium being associated with successful integration of a single threaded screw type implant.	[22]
					Liver	Ti	170	140			
					Lung	Ti	290	120			
					Spleen	Ti	160	200			
Wistar rats	Ti-6Al-4V alloy	n/a	45	6,12,24, 48 w	Tibia	Ti	4.8	0.8	GF-AAS ICP-MS	Minute quantities of Ti, Al and V in the tibia tissues with the Ti-6Al-4V implant were found. The Ti concentration in the tibia tissue with the Ti-15Zr-4Nb-4Ta	[26]
						Al	1.2	0.2			
						V	0.2	0.007			
					Tibia	Ti	1.8	0.8			
						Zr	0.03	0.03			

	Ti-15Zr-4Nb-4Ta alloy					Nb	0.02	0.01		implant was lower than that in the tibia tissues with the Ti-6Al-4V implant. The Zr, Nb and Ta concentrations were also very low. The Ti-15Zr-4Nb-4Ta alloy with its low metal release in vivo is considered advantages for long-term implants.	
						Ta	0.02	0.001			

Analytical techniques: ICP-MS, inductively coupled plasma mass spectrometry; ICP-OES, inductively coupled plasma optical emission spectrometry; GF-AAS, graphite furnace atomic absorption spectrometry ; Abbreviations: TiN-titanium nitride; n/a-data not available; w-weeks; m-months

2.2. Metallic ions in inner organs of animals injected with titanium particles of titanium alloys

Titanium has long been regarded as an inert and biocompatible highly corrosion resistant metal, because of the thin and stable protective oxide layer of TiO_2 spontaneously formed on its surface. However, implanted Ti undergoes events such as bending, scratching or corrosion 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. As a result of corrosion, micro- and nanoparticles can be released into the biological environment, and their effect on tissues is seemingly associated with differences and particular physicochemical properties of titanium, when these biomaterials are used in implantology. In addition, titanium particles from exogenous sources can enter the body through inhalation, ingestion, or dermal contact.

Up till now, most studies have been focused on the biodistribution of titanium(IV) oxide (TiO_2) as nanoparticles in animal organism [27-31]. Giertz et al. [27] have looked for damages in tissues of liver, kidney, lung and heart of rats submitted to injection of Hank's solution containing particles of Ti-6Al-4V alloy, obtained by metal friction. The particle size ranges from 50 to 200 μm for the Ti alloy. Lesions caused by an inflammatory response such as strange body epithelioid, granuloma and giant cells were found in some of the tissues containing yttrium and aluminum. Tissue distribution of various titanium dioxide nanoparticles were investigated in rats up to 90 days post-exposure after intravenous administration of a single or five repeated doses [28]. Both after single and repeated intravenous (IV) exposure, titanium rapidly distributed from the systemic circulation to all tissues evaluated (liver, spleen, kidney, lung, heart, brain, thymus, reproductive organs). Liver was identified as the main target tissue, followed by spleen and lung. The results of intravenous (IV) exposure study indicated slow tissue elimination. Rats exposed orally to various TiO_2 nanoparticles showed no or very low titanium levels in liver and spleen and low titanium levels in mesenteric lymph nodes up to 90 days after exposure, suggesting only minor absorption from the gastro-intestinal tract. Elgrabli et al. [29] have focused on TiO_2 nanoparticle biodistribution, clearance and toxicological effects after intravenous injection, considering TiO_2 translocation in the blood occurs. They found TiO_2 accumulation in liver, lungs and spleen. Authors showed that TiO_2 nanoparticles were quickly eliminated from blood and relocated in liver, spleen and lungs. Here, they showed that TiO_2 nanoparticles could be cleared from the body with a half-life of 12.7 days. Umbreit et al. [30] analyzed the tissue distribution of nano-TiO_2 in mice after the intravenous (56 or 560 mg kg^{-1} per mice) injection. The mice were inspected at 2, 4, 12 and 26 weeks after administration. Over that time microgranulomass (clusters of macrophages and lymphocytes with agglomerated TiO_2) were observed in the main filtering organs: liver, lungs and spleen. Chen et al. [31] have investigated the *in vivo* acute toxicity of nano-sized TiO_2 particles to adult mice. Mice were injected with different dosages of nano-sized TiO_2. The effects of particles on serum biochemical levels were evaluated at various time points (from 24 h to 14 days). The accumulation of TiO_2 was highest in spleen. TiO_2 was also deposited in liver, kidney and lung. This indicated that nano-TiO_2 particles could transport to and deposit in other tissues after intraperitoneal injection.

Two papers presented the method development and the results of the determination of tissue titanium levels after treatment of rats with the nano-sized TiO_2 [32,33]. Total Ti levels were chosen to evaluate the presence and distribution of TiO_2 nanoparticles in tissue (liver, lung, spleen, heart, kidney, brain, muscle) samples taken from rats.

As the biosafety of nanotechnology becomes a growing concern, the *in vivo* nanotoxicity of nanoparticles has been drawn an increasing attention. One approach to detecting TiO_2 nanoparticles is the radioactive tracer method. Xie et al. [34] determined tissue distribution and excretion after intravenous administration of TiO_2 nanoparticles using TiO_2 particles radiolabelled with ^{125}I. Titanium dioxide nanoparticles (TiO_2-NPs) have been developed for versatile use; the TiO_2-NPs were injected in mice or rats with the concentration of 1 mg mL^{-1} and the dose of 10 mg kg^{-1} body weight and their tissue distribution and excretion were investigated by using *ex vivo* fluorescent imaging technique. The results indicated that the TiO_2-NPs mainly accumulated in liver and spleen and could be retained for over 30 days in these tissues. The liver plays a key role in accounting for the rapid accumulation of TiO_2 particles after exposure *via* the intravenous route. The excretion assay found that the excretion rate of TiO_2-NPs through urine was higher than that of feces, indicating that renal excretion was the main excretion pathway of TiO_2-NPs. Overall results of the present study provided important information on distribution and excretion of TiO_2-NPs *in vivo* [34].

The kinetics of nano-sized titanium dioxide in rats after repeated 13-weeks oral exposure was studied by Cho et al. [35]. Titanium dioxide particles (commercially available, primary particles size of 26.4 ± 6.1 nm) were administered to rats in doses of approximately 250, 500 and 1000 mg kg^{-1} bw/day, 7 days/week for 13 weeks. No clear dose-related increases in titanium levels in liver, spleen, kidney and brain could be observed, indicating very low systemic bioavailability. At high dose levels such as several hundreds to one thousand milligram/kg bw/day, absorption of titanium dioxide might be reduced due to agglomeration/aggregation of the particles in the gastrointestinal track. The low absorption of TiO_2 nanoparticles might be favorable, especially for no nutritional application.

Imagining technique, X-ray fluorescence (XRF) was used for *in vivo* investigation of distribution and accumulation of nanoparticles-TiO_2 in lungs [36,37], the olfactory bulb [38] and brain [39] of mice after intratracheal instillation. After intranasal instillation of TiO_2 nanoparticles, the concentration in cerebral cortex (approximately 100 ng g^{-1}) was significantly higher in the instillation group than in the control group due to translocation to the brain *via* olfactory bulb [39].

In order to evaluate the toxicity of TiO_2 particles, the acute toxicity of nano-sized TiO_2 particles (25 and 80 nm) on adult mice was investigated compared with fine TiO_2 particles (155 nm). A fixed large dose of 5 g kg^{-1} body weight of TiO_2 suspensions was administrated by single oral gavages [40]. TiO_2 80-nm nanoparticles mainly retained in liver, kidneys, spleen, and lung by determining the titanium content; the liver and kidneys of mice were slightly affected by TiO_2 nanoparticles after the acute exposure at a single 5 mg kg^{-1} gastric gavage dose, which indicated that TiO_2 particles could be transported to other tissues and organs after by gastrointestinal tract.

Sarmiento-González et al. [24] has been established 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. A comparative study of the systemic distribution from Ti implants was also carried out by intraperitoneally injection of soluble Ti(citrate), and insoluble TiO_2 particles, respectively. Different systemic Ti storage was observed. Whereas soluble Ti was rapidly transported to all distal organs under study, TiO_2 particles were only accumulated in lung tissue. The Ti distribution pattern observed for soluble Ti when intraperitoneally injected as Ti(citrate), 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 [41].

Most studies have focused on the biodistribution of titanium(IV) oxide as nanoparticles or crystals in organism. But, as Sarmiento-González et al. [24] suggested, soluble Ti ions are the main degradation products of these metal implants. Moreover, metal in such form has shown higher toxicity to cell lines than the nanoparticles at similar concentration [42]. In another study, Golasik et al. [43] investigated the titanium distribution in the liver, spleen and kidneys of rats following single intravenous or 30-days oral administration for metal (6 mg Ti kg^{-1}/b w) in ionic form. Titanium was mainly retained in kidneys after both intravenous and oral dosing, and also its compartmentalization in this organ was observed. Titanium in the liver was non-uniformly distributed-metal accumulated in single aggregates, and some of them were also enriched in calcium. Correlation analysis showed that metal did not displace essential elements, and in liver titanium strongly correlated with calcium. Accumulation of Ti in kidney, liver and spleen was observed after long-term exposure which can simulate this situation in patients with titanium implants.

In a series of papers, Olmedo research group [44-49] study the distribution, destination, and potential risk of titanium nano-sized particles administered subcutaneously or intraperitoneally to rats. Some of those studies focused simply on identification of Ti particles in target organs by use of the electron microprobe. Injection of TiO_2 resulted in widespread distribution of the material in the abdominal area, with deposits in liver, spleen and lung. TiO_2 particles and Ti(citrate), were injected in one single dose, ranging from $1.6x10^6$ to $1.6x10^8$ ng Ti. The authors studied the effect of TiO_2 on the oxidative metabolism of alveolar macrophages. They attributed the generation of reactive oxygen species (ROS) to an adaptive response to TiO_2 particles, because they failed to observe any tissue damage 18 months after injection. The histological analysis of TiO_2-exposed rats revealed the presence of abundant intracellular aggregates of metallic particles of Ti in peritoneum, liver, lungs and spleen at the higher dose 16,000 mg kg^{-1}, 5 month after the injection. It was also reported those 6 months after intraperitoneal injection, 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). It was demonstrated that TiO_2 is not only transported bound to plasma proteins in the blood but is also transported associated to cells of the phagocytic-mononuclear lineage. Their results showed a direct relationship between the size of the particles and the presence of alterations in the lungs, liver, and kidneys, and that nanoparticles (NPs) were more harmful than the microparticles (MPs). Titanium concentration varied with particle size in both liver and lung samples 1 month post-injection, and was higher in samples from rats injected with MPs.

In another study, tissue distribution of TiO_2 nanoparticles (20-30 nm) was assessed in rats following intravenous injection (5 mg kg^{-1} b/w) 1, 14 and 28 days post exposure [50]. TiO_2 nanoparticles were cleared from blood and primarily accumulated within liver, with Ti also found in spleen, kidneys, and a small amount translocated to the lungs. TiO_2 levels within the liver were still elevated at the last exposure time point (28 days). Translocation to the brain was below the detection limit (<500 μg/organ). However, levels decreased within time in the other organs. No serum cytokine or enzyme changes were observed.

Sinohara el al. [51] investigated the organ-tissue distribution, after exposure *via* the various routes, and clearance over time of TiO_2 nanoparticles (P25) to rats (0.95 mg kg^{-1} b/w). Blood concentrations of TiO_2 were 420 and 19 μg L^{-1} at 5 and 15 min after administration. At 6 h of administered TiO_2 was found in the liver, spleen, lung, kidney, heart and blood. Liver and spleen TiO_2 burden was significantly higher in the administration than control group and did not decrease up to 30 days after administration, while TiO_2 burden in the lung, kidney, and heart and blood decreased over time. Ti content in faces and urine in the TiO_2 administration group did not differ from that in the control group.

The total concentrations of Ti in the inner organ tissues of animals following injection of titanium suspension are presented in Table 3.

3. Cobalt and chromium ions release from cobalt-based alloy implants

Cobalt-based biocompatible alloy has a unique combination of properties which have enabled it to be a well established implant material. The cobalt-based alloys exhibit balance between biocompatibility and mechanical properties. The wear resistance of Co alloys is higher than that of both Ti alloys and stainless steel alloys. However, literature information concerning the ion levels such as Co and Cr in tissues associated with the use of cobalt-based implants is limited. Comparison between studies is difficult, due to the use of different animal models and the lack of a common procedure for implantation used in each study.

In 1983, Woodman et al. [52] investigated cobalt and nickel levels in the serum, erythrocytes, and periarticular tissue (muscle from the sartorius and vastus medialis) resulting from the implantation of total finger joint prosthesis made of vitallium (Co-Cr-Mo alloy) in the knee of the cat for periods of up to one year. The results indicated significant elevation in serum cobalt concentrations only after 5 months and increased concentrations in serum nickel which, were more pronounced after two months. Column chromatographic separations show a different distribution across the three principal protein peaks for each metal.

To compare metal concentrations in tibia tissues with various metallic implants, Co-Cr-Mo casting alloy was implanted into the rat tibia for up to 48 weeks [26]. The concentrations of metals in the tibia tissues were determined. The Co concentration in the tibia tissues with the Co-Cr-Mo implant was low, and it increased up to 24 weeks and slightly decreased at 48 weeks. The Cr concentration increased up to 12 weeks and decreased toward 48 weeks. The Cr concentration tended to be higher than the Co concentration. The change in Mo concentration was very small at 6 weeks or later.

Rubio et al. [23] presented 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 material was Co-Cr alloy. The implant was inserted in the hind legs of Wistar rats. Analysis for Co, Cr and Al metallic traces was performed after a long exposure time of 12 months. According to the results, the highest Cr and Al concentrations were detected in spleens. Co is mainly found in kidneys, since this element is eliminated *via* urine. Cr traces increased significantly in rat organs after the long implantation time. 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.

Bilo et al. [53] show that Atomic Layer Deposition is a suitable coating technique to prevent metal diffusion from medical implants. The metal distribution in pig bone tissue with inserted bare and coated Co-Cr alloys was evaluated by means of micro X-ray fluorescence (μ-XRF) mapping. In the uncoated implant, the migration of Co and Cr particles from the bare alloy in the bone tissues is observed just after one month and the number of particles significantly increases after two months. In contrast, no metal diffusion was detected in the implant coated with TiO_2. Instead, a gradient distribution of Co, Cr and Mo was found, from the alloy surface going into the tissue. No significant change was detected after two months of going. As expected, the thicker is the TiO_2 layer, the lower is the metal migration.

Nickel eluted from metallic biomaterials is widely accepted as a major cause of allergies and inflammation. To improve the safety of Co-Cr-Mo alloy implants, new ultralow-Ni Co-Cr-Mo alloys with and without zirconium have been developed, with Ni contents of less than 0.01% [54]. Authors investigated the biocompability of these new alloys *in vivo* by subcutaneously implanting pure Ni, conventional Co-Cr-Mo, ultralow-Ni Co-Cr-Mo, and ultralow-Ni Co-Cr-Mo with Zr wires into the dorsal sides of mice. Therefore, they investigated metal ion release from implanted ultralow-Ni Co-Cr-Mo alloy wires with and without Zr and monitored effects on the expression of inflammatory genes in mice. No inflammatory reactions were observed in the presence of Co-Cr-Mo alloys. However, Ni ion release from ultralow-Ni Co-Cr-Mo, and ultralow-Ni Co-Cr-Mo with Zr was significantly less than that from the conventional Co-Cr-Mo. Finally, inflammation was correlated with Ni ion release from Ni wires.

Table 3 Ti total concentration in inner organ tissues of animals following injection of TiO2 nanoparticles suspension

Animal	Intravenous injection	Injection data		Organ tissue analysed	Metallic traces measured	Concentration ($\mu g\ L^{-1}$/$ng\ g^{-1}$)	Analytical technique	Research results	Refs.
		No. animals	Injection time						
Sprague Dawley rats	1.7 mg TiO_2 in NaCl 0.9% suspensions	6	1 h, 1,7,28,56 d and 10 m	Kidney	Ti	1.34	ICP-OES	Results showed absence of toxicological effects after TiO_2 intervenes injection at concentrations of 7.7 to 9.4 mg kg^{-1}.	[29]
				Brain	Ti	0.37			
				Spleen	Ti	35			
				Lungs	Ti	6.89			
				Liver	Ti	9.39			
				Blood	Ti	0.33			
				Urine	Ti	1.03			
Mice	TiO_2 suspension $[Ti(OC_4H_9)_4]$	70	24,48 h and 7,14 d	Liver	Ti	400	ICP-MS	TiO_2 particles induced some significant pathological changes as passive behavior, tremor and lethargy.	[31]
				Spleen	Ti	1200			
				Lung	Ti	100			
				Kidney	Ti	80			
Wistar rats	TiO_2 (2.56 mg mL^{-1})	28	14 d	Liver	Ti	118	ICP-MS	Total Ti levels were chosen to evaluate the presences and distribution of TiO_2 nanoparticles.	[32]
				Lung	Ti	17			
				Spleen	Ti	70			
Sprague Dawley rats	TiO_2 nanoparticles aqueous solution (2 $\mu g\ L^{-1}$)	62	n/a	Brain	Ti	5	GF-AAS ICP-MS	The method could be applicable to *in vivo* studies of TiO_2-NPs biokinetics and toxic kinetics studies.	[33]
				Liver	Ti	21			
				Spleen	Ti	3			
				Kidney	Ti	7			
				Lung	Ti	4			
				RBC	Ti	5			
				Plasma	Ti	3			

				Urine	Ti	11			
Sprague Dawley rats	TiO_2 nanoparticles aqueous solution ($5\ mg\ mL^{-1}$) Oral administration	11	13 m	Liver	Ti	0.15	ICP-MS	Tissue distribution data showed that TiO_2 nanoparticles were not significantly increased in sampled organs.	[35]
				Spleen	Ti	0.7			
				Kidney	Ti	0.4			
				Brain	Ti	0.8			
				Urine	Ti	1.0			
				Feces	Ti	8000			
Rats	TiO_2NPs disodium phosphate solution ($2\ mg\ mL^{-1}$) Intratracheal administration	3	n/a	Lung	Ti	3.6	XRF	Quantification of the pulmonary microdistribution of TiO_2NPs in lung was established.	[36]
Mice	TiO_2 nanoparticles suspension (500 μg) Nasal instillation	n/a	30 d	Brain	Ti	290	ICP-MS	Results provided the preliminary evidence that nasal instilled TiO_2NPs could be translocated into the central nervous system and cause potential lesion of brain.	[39]
Mice	TiO_2 hydroxy propylmethlcellulose suspension (3 g TiO_2, 0.5% HPMC) Oral administration	80	2 w	Liver	Ti	4000	ICP-MS	TiO_2NPs mainly retained in liver, kidneys and spleen, which indicated that TiO_2 particles could be transported to other tissues and organ after uptake by gastrointestinal track.	[40]
				Spleen	Ti	400			
				Kidney	Ti	390			
				Lung	Ti	500			
				Brain	Ti	150			
Rats	Titanium citrate solution (6 mg Ti)	6	0.5 and 3 h	Liver	Ti	10		Results indicate that μ-SRXRF can provide the Ti in ionic form distribution in whole organs.	[43]
				Spleen	Ti	5			
Wistar rats	TiO_2 saline solution (5 mL,	18 and 26	3,6,18 m	Serum	Ti	66.9	ICP-MS	Results show that biokinetics of TiO_2NPs depends on	[48]
					Zr	46.3			

	1.6 g/100 g) Intraperitoneally injected			Lung	Ti	91.3		differences in physico chemical properties of the particles.	
					Zr	10.3			
				Liver	Ti	684			
					Zr	253			
Wistar rats	Intraperitoneally injected 45 µm TiO_2 or 5 nm TiO_2 in 5 mL 0.9% NaCl suspension	n/a	1 m	Plasma	Ti	1.8-2.0 *mg kg^{-1}*	ICP-MS	Plasma titanium concentrations were significantly higher in the TiO_2-NP5 and TiO_2-MP45 groups as compared with the control group. Titanium concentration varied with particle size in both liver and lung samples.	[49]
				Liver	Ti	100			
				Lung	Ti	6.5			
				Kidney	Ti	36			
Wistar rats	TiO_2 (0.5%) Rat serum mixture	12	1,14,28 d	Liver	TiO_2	133.8	ICP-OES	Rats exposed to TiO_2NPs showed expected tissue distribution, no obvious toxic health effects, no immune response, and no change in organ function.	[50]
				Spleen	TiO_2	78.7			
				Lung	TiO_2	8.8			
				Kidney	TiO_2	0.67			
Rats	TiO_2NPs (2 g) in 50 mL of 0.2% disodium phosphate solution. Supernatant (30 mL) as stock suspension	5	6,24 h and 7,30 d	Blood	TiO_2	420	ICP-OES	The tissue distribution, clearance and excretion of TiO_2NPs after intravenous administration using a highly sensitive analytical method.	[51]
				Lung	TiO_2	400			
				Kidney	TiO_2	32			
				Liver	TiO_2	29			
				Spleen	TiO_2	9000			
				Heart	TiO_2	77			
				Brain	TiO_2	4.8			

Analytical techniques: ICP-MS, inductively coupled plasma mass spectrometry; ICP-OES, inductively coupled plasma optical emission spectrometry; GF-AAS, graphite furnace atomic absorption spectrometry; XRF-X-ray fluorescence; µ-SRXRF-micro synchrotron radiation-induced X-ray fluorescence

Abbreviations: HPMC-hydroxy-propyl methylcellulose; NPs-nanoparticles; RBC-red blood cells; n/a-data not available; h-hours; d-days; w-weeks; m-months

Table 4 Trace metal concentration in inner organ tissues of animals following ion release from cobalt-based alloy implants

Animal	Implant material	Implant specification	Implantation data		Organ tissue analyzed	Metal ion(s) measured	Concentration (µg L^{-1})		Analytical technique	Research results	Refs.
			No. animals	Implantation time			Metal levels	Control levels			
Wistar rats	Co-Cr alloy Co-Ni-Cr-Mo alloy	Cylinders	3	12 m *in vivo*	Kidney	Co	0.6		ICP-MS	The *in vivo* release process is evident from the metal traces detected in the organs of rats with C-Cr alloy implants.	[23]
						Cr	0.51				
					Liver	Co	0.15				
						Cr	0.33				
					Lung	Co	0.15				
						Cr	0.54				
					Spleen	Co	0.11				
						Cr	0.62				
Wistar rats	Co-Cr-Mo casting alloy	Rods	45	6,12,24,48 w	Tibia	Co	1.5		ICP-MS	The chromium concentration tended to be higher than the cobalt concentration.	[26]
						Cr	2.5				
Mice	Ultralow-Ni Co-Cr-Mo alloy	Wires	7	3,7 d	Dorsal skin	Ni	0.01	0.001	ICP-MS	Inflammation was correlated with Ni ion release from Ni wires, suggesting that the present ultralow-Ni Co-Cr-Mo alloys with and without Zr have greater safety, and utility than	[54]
						Co	0.12	0.02			
						Cr	0.01	0.01			
						Mo	0.05	0.05			
	Conventio-nal Co-Cr-Mo alloy					Ni	0.04	0.001			
						Co	0.17	0.02			
						Cr	0.07	0.01			
						Mo	0.08	0.05			
	Ultralow-Ni Co-Cr-					Ni	0.005	0.002			

	Mo alloy with Zr					Co	0.13	0.02		conventional Co-Cr-Mo alloys.	
						Cr	0.01	0.01			
						Mo	0.06	0.05			
Charolais rams	Cast Co-Cr alloy	Femoral stem, head and cup	n/a	1,3,6,9,12 and 13 m	Blood	Co	4.430	1.190	n/a	Evidence of gray tissue staining was observed in the synovial of implants in the MoM group.	[55]
						Cr	0.805	0.240			

Analytical techniques: ICP-MS, inductively coupled plasma mass spectrometry Abbreviations: n/a-data not available; d-days; w-weeks; m-months

Blunn et al. [55] developed an ovine total hip arthroplasty ram model to evaluate metal ion release, wear, the biological response and adverse tissue reaction to metal-on-metal (MoM) bearing materials *in vivo*. The performance of an advanced superlattice ceramic coating (SLC) was evaluated as a bearing surface; MoM articulating surfaces coated with a SLC coating (SLC-MoM) and uncoated MoM surfaces. Implants remained *in vivo* for 13 months and blood chromium and cobalt metal ion levels were measured pre and postoperatively. When compared with the MoM group, sheep with SLC-MoM implants showed significantly lower levels of chromium and cobalt metal ions within blood over the 13-months period. The coating offers the opportunity to increase the survival of joint replacements by reducing metal ion release and may be suitable for patients that have metal ion sensitivities.

Nickel has a number of adverse biological effects that have made the use of nickel in biomedical implants controversial. Ni ions are easily released from solid Ni and produce localized toxicity [56]. Accordingly, Wataha et al. [57] demonstrated ionization of Ni from the surface of implanted wires by body fluid in rats, and high concentrations of Ni ions around the wire resulted in necrosis. Spatial distribution of nickel around nickel-containing implants *in vivo* was assessed. Pure nickel wire or a nickel-containing alloy (Ni-Cr) was implanted subcutaneously into rats for 7 days. The tissues were analyzed for Ni content and inflammation. The Ni-Cr wire caused inflammation and that the nickel distribution in tissues correlated well with overt tissue inflammation.

The concentrations of trace metals in the inner organ tissues of animals following ion release from cobalt-based alloy implants are presented in Table 4.

4. Analysis of metallic traces from magnesium-based alloy implants in animal inner organs

The use of biodegradable magnesium-based metal as implant is one of the latest development in temporary metallic implants changing the custom of only developing highly corrosion resistant metals [58]. Magnesium alloys have been suggested for bone implant application due to their low density, inherent biocompatibility, perfect mechanical properties, and a promising candidate for biodegradable bone implants. Animal models have been widely used in biomedical research and have been crucial for acquiring basic science and clinical knowledge pertaining to medical science. Several researchers studied the *in vivo* behavior of magnesium based materials implanted in humans as well as in experimental animals [59, 60]. Animal studies on bone defect repair with biodegradable magnesium and magnesium alloys in comparison with other traditional biomaterials were reviewed [61, 62].

Witte et al. [63] have investigated the degradation mechanism as the bone-implant interface of different degrading magnesium alloys in bone and determined their effect on the surrounding bone. Sample rods of four different magnesium alloys were implanted intramedullary into the femora of guinea pigs. After 6 and 18 weeks, the bone-implant interface was characterized in uncalcified sections. Results showed that metallic implants made of magnesium alloys degrade *in vivo* depending on the composition of the alloying elements.

Rare earth element (REE) concentration gradients in bone were quantitatively investigated [64]. The maximum concentration of REE in bone around biologically degradable magnesium alloy implant was ~200 μg g^{-1} after six weeks of implantation compared to background concentration of 3-4 μg g^{-1}. No further accumulation of degraded REE in bone tissue around the implant was detected after 12 weeks of implantation. New bone formation was identified by a variable Ca/P-ratio.

Xu et al. [65] have investigated the *in vivo* degradation behavior of Mg-Mn-Zn alloys to assess the feasibility of Mg-Mn-Zn alloys for use as bone implant materials; magnesium alloy has been implanted in rats. After 9 weeks post operation, a degradation of reaction layer, which was mainly composed of Ca, P, O, and Mg, was formed on the surface of magnesium alloy implants. As Mg is released gradually, and Mg levels in the blood are regulated by the kidneys, the excess is excreted in urine. About 54% magnesium implant has degraded *in vivo*. Element analysis showed that Zn and Mn in Mg-Mn-Zn alloy distributed homogeneously in the residual magnesium implant, the degradation layer, and the surrounding bone tissue after 18 weeks implantation, indicating that Zn and Mn elements were easily absorbed by bioenvironmental.

Angrisani et al. [66] have investigated the degradation process of a magnesium alloy *in vivo* for up to 3.5 years after implantation of cylindrical pins into the medullary cavity of New Zealand White rabbits. The degradation process of magnesium based implants was slow and homogenous. Total amounts of rare elements (RE) detected in the inner organs were very low: the organs of rabbits with magnesium based cylinders showed 10-20-fold increased concentrations.

Table 5 Metallic traces concentration in inner organ tissues of animals from magnesium-based alloy implants

Animal	Implant material	Model	Implantation data		Organ tissue analyzed	Metallic traces measured	Concentration (μg g^{-1})	Analytical technique	Research results	Refs.
			No. animals	Implantation time						
White rabbits	Magnesium alloy	Femoral condyle	n/a	2,6,12 w	Bone	La	50-100	LA-ICP-MS	No accumulation of degraded trace elements from the magnesium implants in the organic part of the adjacent-bone was observed.	[64]
						Ce	90-400			
						Pr	10-25			
						Nd	40-100			
White rabbits	LAE442-based magnesium alloy	Cavity tibiae	8	9 m and 3.5 y *in vivo*	Liver	Al	1.8	ICP-OES	Total amounts of RE detected in the inner organs were very low, the organs of rabbits with LAE442 cylinders showed 10-20-fold increased concentrations of the alloying elements lanthanum, cerium, neodymium and praseodymium compared to animals without any implanted material.	[65]
						AlLa	0.550.397			
						Ce	0.894			
						Pr	0.075			
						Nd	0.208			
					Kidney	La	0.007			
						Ce	0.020			
						Pr	0.002			
					Spleen	Nd	0.008			
						Al	1.8			
					Brain	La	0.397			
						Ce	0.394			
						Pr	0.075			
						Nd	0.208			
						Al	0.07			
						La	0.011			

						Ce	0.023			
						Pr	-			
						Nd	0.009			
Wistar rats	AZ31-based magnesium alloy	Femur fracture	36	1,9,13 m	Organ tissues (Kidneys, livers, lungs, spleens, brains)	Mg	641	ICP-MS	Aluminum is the element that requires special attention.	[67]
						Zn	65			
						Mn	3			
						Al	5			

Analytical techniques: LA-ICP-MS, laser ablation inductively coupled plasma mass spectrometry; ICP-MS, inductively coupled plasma mass spectrometry; ICP-OES, inductively coupled plasma optical emission spectrometry Abbreviations: n/a-data not available; w-weeks; m-months; y-years

Bodelón et al. [67] have evaluated metallic traces such as Mg, Al, Zn, Mn and, in addition, fluorine (F) in different rat organs, such as the liver, kidneys, spleen, lungs and brain, resulting from the biodegradation process of magnesium (AZ31) alloy intramedullary pins in Wistar rat femurs. The main conclusions and the clinical relevance of the study have been that AZ31 endomedullary implants have a degradation rate of about 60% after 13 months, which is fully compatible with fracture consolidation. Neither bone fracture nor an MgF_2 coating seems to influence the accumulation of trace elements in the studied organs. Aluminum is the only alloying element in this study that requires special attention. The increase in Al recovered from the sampled organs represents 3.95% of the amount contained in the AZ31 implant.

Metallic traces concentration in the inner organ tissues of animals with magnesium-based implants are presented in Table 5.

5. Conclusion

Animal models have been widely used in biomedical research and have been crucial for acquiring basic science and clinical knowledge pertaining to medical science. The most commonly used laboratory animals have turned out to be rats, mice, and rabbits, probably because they are cheaper and easy to handle. However, use of small and cheap animals could be accepted in the early stages of the test, whereas the healing characteristics of the animals should approximate those of humans during the late stages. Although, there is no animal model presenting with the same anatomic, biochemical, physiologic, and biological characteristics as those found in human beings. Useful data for treating orthopedic patients are based not only on good planning and study design, but also on perfect knowledge of the animal used and of the differences between the model and the human being. Biomedical implants should be subjected to both *In vitro* and *in vivo* studies for their application. *In vitro* studies which are performed in simulated body condition give an overview of the behavior of the material under the given condition and obviously it cannot be taken as the final test to recommend a material as an implant. Regarding the *in vivo* test, there is no consensus about the appropriate animal model that can provide a clear indication of the systemic effects that metallic ions can cause in the human being. The *in vivo* tests which are performed using animal models evaluate the actual performance of the materials and these tests are required, in order that it is approved by health agencies. Various analytical methods, i.e. GF-AAS, ICP-OES, LA-ICP-MS have been used to determine trace and ultra trace elements in animal tissues, body fluids and inner organ tissues. Given the implicit and growing need for sensitivity, the choice of the instrument is of primary importance. The use of ICP-MS of latest generation would allow decreasing easily the limit of detection and quantification. As a consequence, the determination of very low metal levels in animal models has become a priority, and analytical/animal specimen methodologies have had to improve to meet demanding expectations for accuracy, precision, analysis time and multi metal assays in clinical diagnostics and disease research.

Compliance with ethical standards

Acknowledgments

This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

Disclosure of conflict of interest

All authors declare no conflicts of interest associated with this manuscript.

References

[1] Sarmiento-González A, Marchante-Gayón JM, Tejerina-Lobo JM, Paz-Jiménez J, Sanz-Medel A, High-resolution ICP-MS determination of Ti, V, Cr, Co, Ni, and Mo in human blood and urine of patients implanted with a hip or knee prosthesis. Analytical and Bioanalytical Chemistry. 2008; 391(7): 2583-9.

[2] Bagchi D, Stohs SJ, Downs BW, Bagchi M, Preuss HG, Cytotoxicity and oxidative mechanisms of different forms of chromium. Toxicology. 2002; 180(1): 5-22.

[3] Fini M, Giardino R, *In vitro* and *in vivo* test for the biological evaluation of candidate orthopedic materials: Benefits and limits. Journal of Applied Biomaterials and Biomechanics. 2003; 1: 155-63.

[4] Manivasagam G, Dhinasekaran D, Rajamanickam A. Biomedical implants: Corrosion and its prevention - A Review. Recent Patents on Corrosion Science. 2010; 2: 40-54.

[5] Giardino R, Fini M, Giavaresi G, Experimental surgery in the research of artificial organs. International Journal of Artificial Organs. 1998; 21(9): 506-8.

[6] Okazaki Y, Gotoh E, Comparison of metal release from various metallic biomaterials *In vitro*. Biomaterials. 2005; 26: 11-21.

[7] Ferguson Jr. AB, Laing PG, Hodge ES, The ionization of metal implants in living tissues. Journal of Bone and Joint Surgery Am. 1969; 42-A: 77-90.

[8] Ferguson Jr. AB, Akahoshi Y, Laing PG, Hodge ES. Characteristics of trace ions released from embedded metal implants in the rabbit. Journal of Bone and Joint Surgery Am. 1962; 44- A(2): 323-3.

[9] Ferguson Jr. AB, Akahoshi Y, Laing PG, Hodge ES, Trace metal ion concentration in the liver, kidney, spleen, and lung of normal rabbits. Journal of Bone and Joint Surgery Am. 1962; 44-A(2): 317-22.

[10] Laing PG, Ferguson Jr. AB, Hodge ES, Tissue reaction in rabbit muscle exposed to metallic implants. Journal of Biomedical Materials Research. 1967; 1(1): 135-49.

[11] Woodman JL, Jacobs JJ, Galante JO, Urban RM, Metal ion release from titanium-based prosthetic segmental replacements of long bones in baboons: A Long-term study. Journal of Orthopaedic Research. 1984; 1: 421-30.

[12] Seki K, Elution and biocompatibility of titanium in the rabbit tissue. Dent Journal of Iwate Medical University. 1992; 17: 158-77.

[13] Bianco PD, Ducheyne P, Cuckler JM, Local accumulation of titanium released from a titanium implant in the absence of wear. Journal of Biomedical Materials Research. 1996; 31: 227-34.

[14] Bianco PD, Ducheyne P, Cuckler JM, Titanium serum and urine levels in rabbits with a titanium implant in the absence of wear. Biomaterials. 1996; 17(20): 1937-42.

[15] Bianco PD, Ducheyne P, Cuckler JM, Systemic titanium levels in rabbits with a titanium implant in the absence of wear. Journal of Materials Science: Materials in Medicine. 1997; 8: 525-9.

[16] Ektessabi AM, Otsuka T, Tsuboi Y, Yokoyama K, Albrektsson T, Sennerby L, Johansson C, Application of micro beam PIXE to detection of titanium ion release from dental and orthopaedic implants. International Journal of PIXE. 1994; 4(02n03): 81-6.

[17] Lugowski SJ, Smith DC, McHugh AD, Van Loon JC, Release of metal ions from dental implant materials *in vivo* : Determination of Al, Co, Cr, Mo, Ni, V, and Ti in organ tissue. Journal of Biomedical Materials Research. 1991; 25(12): 1443-58.

[18] Rodriguez D, Gil FJ, Planell JA, Jorge E, Alvarez L, Garcia R, Larrea M, Zapata A, Titanium levels in rats implanted with Ti6Al4V treated samples in the absence of wear. Journal of Materials Science: Materials in Medicine. 1999; 10(12): 847-51.

[19] Lugowski SJ, Smith DC, Van Loon JC, Critical aspects of trace element analysis of tissue samples: A review. Clinical Materials. 1990; 6(2): 91-104.

[20] Mu Y, Kobayashi T, Tsuji K, Sumita M, Hanawa T, Causes of titanium release from plate and screws implanted in rabbits. Journal of Materials Science: Materials in Medicine. 2002; 13: 583-8.

[21] Mu Y, Kobayashi T, Sumita M, Yamamoto A, Hanawa T, Metal ion release from titanium with active oxygen species generated by rat macrophages *In vitro* . Journal of Biomedical Materials Research. 2000; 49: 238-43.

[22] Frisken KW, Dandie GW, Lugowski S, Jordan G, A study of titanium release into body organs following the insertion of single threaded implants into the mandibles of sheep. Australian Dental Journal. 2002; 47(3): 214-7.

[23] Rubio JC, Garcia-Alonso MC, Alonso C, Alobera MA, Clemente C, Munuera L, Escudero ML, Determination of metallic traces in kidneys, livers, lungs and spleens of rats with metallic implants after a long implantation time. Journal of Materials Science: Materials in Medicine. 2008; 19: 369-75.

[24] Sarmiento-González A, Encinar JR, Marchante-Gayón JM, Sanz-Medel A, 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. Analytical and Bioanalytical Chemistry. 2009; 393: 335-43.

[25] Okazaki Y, Nishimura E, Nakada H, Kobayashi K, Surface analysis of Ti-15Zr-4Nb-4Ta alloy after implantation in rat tibia. Biomaterials. 2001; 22(6): 599-607.

[26] Okazaki Y, Gotoh E, Manabe T, Kobayashi K, Comparison of metal concentrations in rat tibia tissues with various metallic implants. Biomaterials. 2004; 25: 5913-20.

[27] Giertz CR, Fernandes BL, Fernandes CR, Franco CRC, Metallic ions in organs of rats injected with metallic particles of stainless steel 316L and Ti6Al4V alloy. Materials Research. 2010; 13(1): 35-9.

[28] Geraets L, Oomen AG, Krystek P, Jacobsen NR, Wallin H, Laurentie M, Verharen HW, Brandon EFA, Win H de Jong, Tissue distribution and elimination after oral and intravenous administration of different titanium dioxide nanoparticles in rats. Particle and Fibre Toxicology. 2014; 11:30: 1-21.

[29] Elgrabli D, Beaudouin R, Jbilou N, Floriani M, Pery A, Rogerleux F, Lacroix G, Biodistribution and clearance of TiO_2 nanoparticles in rats after intravenous injection. PLOS ONE. 2015; 10(4): 1-13.

[30] Umbreit TH, Francke-Carroll S, Weaver JL, Miller TJ, Goering PL, Sadrich N, Stratmeyer ME, Tissue distribution and histopathological effects of titanium dioxide nanoparticles after intravenous or subcutaneous injection in mice. Journal of Applied Toxicology. 2012; 32(5): 350-7.

[31] Chen J, Dong X, Zhao J, Tang G, *In vivo* acute toxicity of titanium dioxide nanoparticles to mice after intraperitioneal injection. Journal of Applied Toxicology. 2009; 29(4): 330-7.

[32] Krystek P, Tentschert J, Nia Y, Trouiller B, Noël L, Goetz ME, Papin A, Luch A, Guérin T, Wim H. de Jong, Method development and inter-laboratory comparison about the determination of titanium from titanium dioxide nanoparticles in tissues by inductively coupled plasma mass spectrometry. Analytical and Bioanalytical Chemistry. 2014; 406: 3853-61.

[33] Devoy J, Brun E, Cosnefroy A, Disdier C, Melczer M, Antoine G, Chalansonnet M, Mabondzo A, Mineralization of TiO_2 nanoparticles for the determination of titanium in rat tissues. Journal of Analytical Chemistry. 2016; 71(4): 418-25.

[34] Xie G, Wang C, Sun J, Zhong G, Tissue distribution and excretion of intravenously administered titanium dioxide. Toxicology Letters. 2011; 205(1): 55-61.

[35] Cho W-S, Kang B-C, Lee JK, Jeong J, Che J-H, Comparative absorption, distribution, and excretion of titanium dioxide and zinc oxide nanoparticles after repeated oral administration. Particle and Fibre Toxicology. 2013; 10(9): 1-9.

[36] Zhang G, Shinohara N, Kano H, Senoh H, Suzuki M, Sasaki T, Fukushima S, Gamo M, Quantitative evaluation of the pulmonary mircrodistribution of TiO_2 nanoparticles using X-ray fluorescence microscopy after intratracheal administration with a microsprayer in rats. Journal of Applied Toxicology. 2015; 35(6): 623-30.

[37] Zhang G, Shinohara N, Kano H, Senoh H, Suzuki M, Sasaki T, Fukushima S, Gamo M, Quantitative evaluation of local pulmonary distribution of TiO_2 in rats following single or multiple intratracheal administrations of TiO_2 nanoparticles using X-ray fluorescence microscopy. Journal of Applied Toxicology. 2016; 36(10): 1268-75.

[38] Wang JX, Chen CY, Yu HW, Sun J, Li B, Li YF, Gao YX, He W, Huang YY, Chai ZF, Zhao YL, Deng XY, Sun HF, Distribution of TiO_2 particles in the olfactory bulb of mice after nasal inhalation using microbeam SRXRF mapping techniques. Journal of Radioanalytical and Nuclear Chemistry. 2007; 272(3): 527-31.

[39] Wang J, Chen C, Liu Y, Jiao F, Li W, Lao F, Li Y, Ge C, Li B, Zhou G, Gao Y, Chai Z, Zhao Y, Potential neurological lesion after nasal instillation of TiO_2 nanoparticles in the anatase and rutile crystal phases. Toxicology Letters. 2008; 183(1-3): 72-80.

[40] Wang J, Zhou G, Chen C, Yu H, Wang T, Ma Y, Jia G, Gao Y, Li B, Sun J, Li Y, Jiao F, Zhao Y, Chai Z, Acute toxicity and biodistribution of different sized titanium dioxide particles in mice after oral administration. Toxicology Letters. 2007; 168: 176-85.

[41] Tinoco AD, Valentine AM, Ti(IV) Binds to human serum transferrin more tightly than does Fe(III), Journal of American Chemical Society. 2005; 127(32): 11218-9.

[42] Soto-Alvaredo J, Blanco E, Bettmer J, Hevia D, Sainz RM, López Cháves C, Sánches C, Llopis J, Sanz-Medel A, Montes-Bayón M, Evaluation of the biological effect of Ti generated debris from metal implants: ions and nanoparticles. Metallomics. 2014; 6(9): 1702-8.

[43] Golasik M, Wrobel P, Olbert M, Nowak B, Czyzycki M, Librowski T, Lankosz M, Piekoszewski W, Does titanium in ionic form display a tissue-specific distribution? Biometals. 2016; 29: 487-94.

[44] Olmedo DG, Tasat DR, Guglielmotti MB, Cabrini RL, Effect of titanium dioxide on the oxidative metabolism of alveolar macrophages: An experimental study in rats. Journal of Biomedical Materials Research. 2005; 73A: 142-9.

[45] Olmedo DG, Tasat DR, Guglielmotti MB, Cabrini RL, Biodistribution of titanium dioxide from biologic compartments. Journal of Materials Science: Materials in Medicine. 2008; 19: 3049-56.

[46] Olmedo DG, Tasat DR, Guglielmotti MB, Cabrini RL, Titanium transport through the blood stream. An experimental study on rats. Journal of Materials Science: Materials in Medicine. 2003; 14: 1099-103.

[47] Olmedo D, Guglielmotti MB, Cabrini RL, An experimental study of the dissemination of titanium and zirconium in the body. Journal of Materials Science: Materials in Medicine. 2002; 13: 793-6.

[48] Olmedo DG, Tasat DR, Evelson P, Rebagliatti R, Guglielmotti MB, Cabrini RL, *In vivo* comparative biokinetics and biocompatibility of titanium and zirconium microparticles. Journal of Biomedical Materials Research. 2011; 98A: 604-13.

[49] Domingo MG, Kurtz M, Maglione G, Martin M, Brites F, Tasat DR, Olmedo DG, Systemic effect of TiO_2 micro- and nanoparticles after acute exposure in a murine model. Journal of Biomedical Materials Research B Applied Biomaterials. 2022; 110(7): 1563-72.

[50] Fabian E, Landsiedel R, Ma-Hock L, Wiench K, Wohlleben W, van Ravenzwaay B, Tissue distribution and toxicity of intravenously administered titanium dioxide nanoparticles in rats. Archives Toxicology. 2008; 82: 151-7.

[51] Shinohara N, Danno N, Ichinose T, Sasaki T, Fukui H, Honda K, Gamo M, Tissue distribution and clearance of intravenously administered titanium dioxide (TiO_2) nanoparticles. Nanotoxicology. 2014; 8(2): 132-41.

[52] Woodman JL, Black J, Nunamaker DM, Release of cobalt and nickel from a new total finger joint prosthesis made of vitallium. Journal of Biomedical Materials Research. 1983; 17(4): 655-8.

[53] Bilo F, Borgese L, Prost J, Rauwolf M, Turyanskaya A, Wobrauschek P, Kregsamer P, Streli C, Pazzaglia U, Depero L, Atomic layer deposition to prevent metal transfer from implants: An X-ray fluorescence study. Applied Surface Science. 2015; 359: 215-20.

[54] Sonofuchi K, Hagiwara Y, Koizumi Y, Chiba A, Kawano M, Nakayama M, Ogasawara K, Yabe Y, Itoi E, Quantitative *in vivo* biocompatibility of new ultralow-Nickel Cobalt-Chromium-Molybdenum alloys. Journal of Orthopaedic Research. 2016; 34: 1505-13.

[55] Blunn GW, Ferro De Godoy R, Meswania J, Briggs TWR, Tyler P, Hargunani R, Wilson H, Khan I, Marriott T, Coathup M, A novel ceramic coating for reduced metal ion release in metal-on-metal hip surgery. Journal of Biomedical Materials Research Part B. 2019; 107B: 1760-71.

[56] Hirasawa N, Goi Y, Tanaka R, Ishihara K, Ohtsu H, Ohuchi K, Involvement of prostaglandins and histamine in nickel wire-induced acute inflammation in mice. Journal of Biomedical Materials Research. 2010; 93(4): 1306-11.

[57] Wataha JC, O'Dell NL, Singh BB, Ghazi M, Relating nickel-induced tissue inflammation to nickel release *in vivo* . Journal of Biomedical Materials Research. 2001; 58(5): 537-44.

[58] Witte F, Hort N, Vogt C, Cohen S, Kainer KU, Willumeit R, Feyerabend F, Degradable biomaterials based on magnesium corrosion. Current Opinion in Solid State and Materials Science. 2008; 12: 63-72.

[59] Witte F, The history of biodegradable magnesium implants: A review. Acta Biomaterialia. 2010; 6(5): 1680-1692.

[60] Zhao D, Witte F, Lu F, Wang J, Li J, Qin L, Current status on clinical applications of magnesium-based orthopaedic implants: A review from clinical translational perspective. Biomaterials. 2017; 112: 287-302.

[61] Zhang J, Jiang Y, Shang Z, Zhao B, Jiao M, Liu W, Cheng M, Zhai B, Guo Y, Liu B, Shi X, Ma B, Biodegradable metals for bone defect repair: A systematic review and meta-analysis based on animal studies. Bioactive Materials. 2021; 6: 4027-52.

[62] Cho DH, Avey T, Nam KH, Dean D, Luo AA, *In vitro* and *in vivo* assessment of squeeze-cast Mg-Zn-Ca-Mn alloys for biomedical applications. Acta Biomaterialia. 2022; 150: 442-55.

[63] Witte F, Kaese V, Haferkamp H, Switzer E, Meyer-Lindenberg A, Wirth CJ, Windhagen H, *In vivo* corrosion of four magnesium alloys and the associated bone response. Biomaterials. 2005; 26:3557-63.

[64] Gruhl S, Witte F, Vogt J, Vogt C, Determination of concentration gradients in bone tissue generated by a biologically degradable magnesium implant. Journal of Analytical Atomic Spectrometry. 2009; 24: 181-8.

[65] Xu L, Yu G, Zhang E, Pan F, Yang K, *In vivo* corrosion behavior of Ma-Mn-Zn alloy for bone implant application. Journal of Biomedical Materials Research Part A. 2007; 83A(3): 703-11.

[66] Angrisani N, Reifenrath J, Zimmermann F, Eifler R, Meyer-Lindenberg A, Vano-Herrera K, Vogt C, Biocompatibility and degradation of LAE442-based magnesium alloys after implantation of up to 3.5 years in rabbit model. Acta Biomaterialia. 2016; 44: 355-65.

[67] Bodelón OG, Iglesias C, Garrido J, Clemente C, García-Alonso MC, Escudero ML, Analysis of metallic traces from the biodegradation of endomedullary AZ31 alloy temporary implants in rat organs after long implantation times. Biomedical Materials. 2015; 10(4): 1-29.