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In vivo comparative biokinetics and biocompatibility of titanium and zirconium microparticles

Daniel G. Olmedo,[1] Deborah R. Tasat,[2] Pablo Evelson,[3] Raúl Rebagliatti,[4] María B. Guglielmotti,[1] Rómulo L. Cabrini[5]

[1]Department of Oral Pathology, School of Dentistry, University of Buenos Aires and National Research Council (CONICET), Buenos Aires, Argentina
[2]School of Science and Technology, National University of San Martín and Department of Histology and Embryology, School of Dentistry, University of Buenos Aires, Argentina
[3]General and Inorganic Chemistry Division, School of Pharmacy and Biochemistry, University of Buenos Aires and National Research Council (CONICET), Buenos Aires, Argentina
[4]Chemical Activity Unit, National Atomic Energy Commission, Buenos Aires, Argentina
[5]Department of Oral Pathology, School of Dentistry, University of Buenos Aires and Department of Radiobiology, National Atomic Energy Commission, Buenos Aires, Argentina

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Abstract: Titanium and zirconium are biomaterials that present a layer of titanium dioxide (TiO_2) or zirconium dioxide (ZrO_2). As a result of corrosion, microparticles can be released into the bioenvironment, and their effect on tissues is seemingly associated with differences in the physicochemical properties of these metals. The aim of this study was to perform a long-term evaluation of the distribution, destination, and potential risk of TiO_2 and ZrO_2 microparticles that might result from the corrosion process. Wistar rats were i.p. injected with an equal dose of either TiO_2 or ZrO_2 suspension. The following end-points were evaluated at 3, 6, and 18 months: (a) the presence of particles in blood cells and liver and lung tissue, (b) Ti and Zr deposit quantitation, (c) oxidant–antioxidant balance in tissues, and (d) O_2^- generation in alveolar macrophages. Ti and Zr particles were detected in blood mononuclear cells and in organ parenchyma. At equal doses and times postadministration, Ti content in organs was consistently higher than Zr content. Ti elicited a significant increase in O_2^- generation in the lung compared to Zr. The consumption of antioxidant enzymes was greater in the Ti than in the Zr group. The present study shows that the biokinetics of TiO_2 and ZrO_2 depends on particle size, shape, and/or crystal structure. J Biomed Mater Res Part A: 98A: 604–613, 2011.

Key Words: titanium, zirconium, microparticles, biokinetics, macrophages

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INTRODUCTION

Biomaterials are widely used in orthopaedic, dental, cardiovascular, ophthalmological, and reconstructive surgery, among other applications. One of the most frequently used metallic biomaterials is titanium.[1] Although zirconium is not widely used as a clinical material, it is chemically closely related to and has several properties in common with titanium.[2] Indeed, the use of zirconium and zirconium alloys to manufacture implants for traumatological, orthopedic, and dental applications has been reported.[3,4]

Titanium and zirconium are highly reactive metals, and, when exposed to fluid media or air, they quickly develop a layer of titanium dioxide (TiO_2) or zirconium dioxide (ZrO_2). This layer of dioxide forms a boundary at the interface between the biological medium and the metal structure. It produces passivation of the metal, determining the degree of biocompatibility and the biological response to the implant.[5–7] TiO_2 exists naturally, mainly in the form of three crystalline structures: rutile, anatasa, and brookite. In the case of titanium implants, the passive oxide layer is composed of anatase and rutile or anatase alone.[8–10] Zirconium, however, does not exist as a free metal in nature; it occurs as the minerals zircon or zirconium silicate ($ZrSiO_4$) and the rare mineral baddeleyite or zirconium dioxide (ZrO_2), which has a monoclinic crystal structure.[11] Baddeleyite, also known as zirconia, is the most naturally occurring form and can be transformed into a tetragonal (1100°C) or cubic (2370°C) crystallographic form depending on temperature.[12,13]

Although both titanium and zirconium are transition metals, their physicochemical properties such as oxidation velocity, interaction with water, crystalline structure, and transport properties, and/or those of their oxides differ

Correspondence to: D. G. Olmedo; e-mail: dolmedo@argentina.com
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quantitatively;[14] these differences may have an effect on biological response.[2]

The TiO_2 or ZrO_2 layer of the implant is subjected to prolonged exposure to the internal electrolyte milieu in the peri-implant biological compartment. All the metallic materials used in surgery as permanent implants are liable, to a certain degree, to corrosion due to variations in the internal electrolyte milieu.[15] Corrosion, one of the possible causes of implant failure implies the dissolution of the protective oxide layer. When metal particles/ions are released from the implant surface, they can migrate systemically, remain in the intercellular spaces near the site where they were released, or be taken up by macrophages.[16,17] The presence of metallic particles in peri-implant tissues may not only be due to a process of electrochemical corrosion but also to frictional wear or a synergistic combination of the two. Moreover, mechanical disruption during insertion, abutment connection or removal of failing implants have been suggested as possible causes of the release of particles from metal structures.[15,18] The release of particles/ions from the implant to the surrounding biological compartment, their biodistribution in the body, and their final destination are issues that lie at the center of studies on biocompatibility and biokinetics.

Based on the above, the potential adverse effects of ions/particles on tissues would seem to be associated with the different and particular physicochemical properties of titanium and zirconium, when these biomaterials are used in implantology.

Thus, the aim of the present study was to evaluate and compare through time, the distribution, target organs, destination, and potential risk of TiO_2 and ZrO_2 microparticles that may result from the corrosion process of metallic biomaterials used in implants.

MATERIALS AND METHODS

Chemicals

TiO_2 particles (anatase), NBT (nitroblue tetrazolium), phosphate-buffered solution, polyvinylpyrrolidone, tetradecanoylphorbol acetate, thiobarbituric acid, superoxide dismutase (SOD), luminol, 2,2′-azo-bis(2-amidinopropane) (ABAP), trolox, bovine serum albumin, and all histological dyes and reagents used in this work, unless specified elsewhere, were purchased from Sigma-Aldrich, Co. (St. Louis, MO). Zirconium dioxide (ZrO_2) particles were provided by Fluka Chemie AG-Switzerland.

Particle characterization: Scanning electron microscopy and energy-dispersive X-ray spectroscopy

Scanning electron microscopy (SEM) study of both particles was carried out using a Zeiss Supra 40 microscope equipped with a field emission gun. Particles were placed on a conductive carbon tape and analyzed without metal coating. Images were obtained using an in-lens detector and 4 kV acceleration voltage. Particles were chemically identified by energy dispersion spectroscopy using an Oxford Instrument detector.

Animal treatment protocol

Male Wistar rats ($n = 62$) weighing ~100 g were injected intraperitoneally (i.p.) with a suspension of either TiO_2 (anatase) (Group Ti) or ZrO_2 (Group Zr) at a dose of 1.60 g/100 g body weight in 5 mL saline solution. Control animals (Group C) were injected with an equivalent volume of vehicle.

At 3, 6, and 18 months, the following end-points were monitored in each group: (a) presence of particles in blood smears; (b) quantitative evaluation of Ti and Zr particles in serum, liver, and lung; (c) deposits of particles in histological sections of the studied organs; (d) generation of superoxide anion (O_2^-) in lung tissue, and (e) oxidant–antioxidant balance in lung and liver tissue homogenates.

Eighteen animals were assigned to the groups studied at 3 and 6 months ($n = 18$), and 26 rats ($n = 26$) were assigned to the group evaluated at 18 months. The latter group included more animals to compensate for the greater likelihood of death.

The Guidelines of the National Institutes of Health (NIH; NIH Publication No. 85-23, Rev. 1985) and the Statement of Ethics Principles of the School of Dentistry of the University of Buenos Aires [Res (CD) 325/02 and Res (CD) 694/02] for the use and care of laboratory animals were observed.

Titanium and zirconium concentration in serum, liver, and lung

Baseline levels of Ti and Zr in serum were determined in all animals before the administration of the oxides or the vehicle. Baseline levels of Ti and Zr in liver and lung tissue were determined in a separate set of five animals that were sacrificed for this purpose. Ti and Zr concentration in serum and organs was studied by inductively coupled plasma optical emission spectroscopy (ICP-OES) Perkin Elmer Optima 3100.

Histological analysis

Blood samples (3 cm^3) were obtained by intracardiac puncture (20G Terumo-USA needle) before sacrifice at all experimental times. Blood smears were prepared and stained with Safranine to easily identify particles phagocytosed by cells.

After sacrifice, the lungs and livers were processed for histological evaluation. The samples were fixed in 10% formalin and routinely processed for paraffin embedding and staining with Grenacher Carmin to facilitate identification of the phagocytosed material. The samples were treated with picric acid to avoid the possible presence of formalin pigments.

Bronchoalveolar lavage

After 3, 6, or 18 months of treatment, lung bronchoalveolar lavage (BAL) was performed as described by Brain and Frank[19] and modified by Tasat and de Rey.[20] Briefly, the thoracic cavity was partly dissected; the trachea was cannulated with an 18G needle and infused 12 times with 1 mL cold sterile PBS pH: 7.2–7.4. The BAL fluid was immediately centrifuged at 800 g at 4°C for 10 min and resuspended in 5 mL PBS.

Generation of superoxide anion (O_2^-) in alveolar macrophages

Superoxide anion (O_2^-), a main reactive oxygen species (ROS) generated during the respiratory burst, was evaluated using the NBT reduction test.[21] BAL cells were treated with

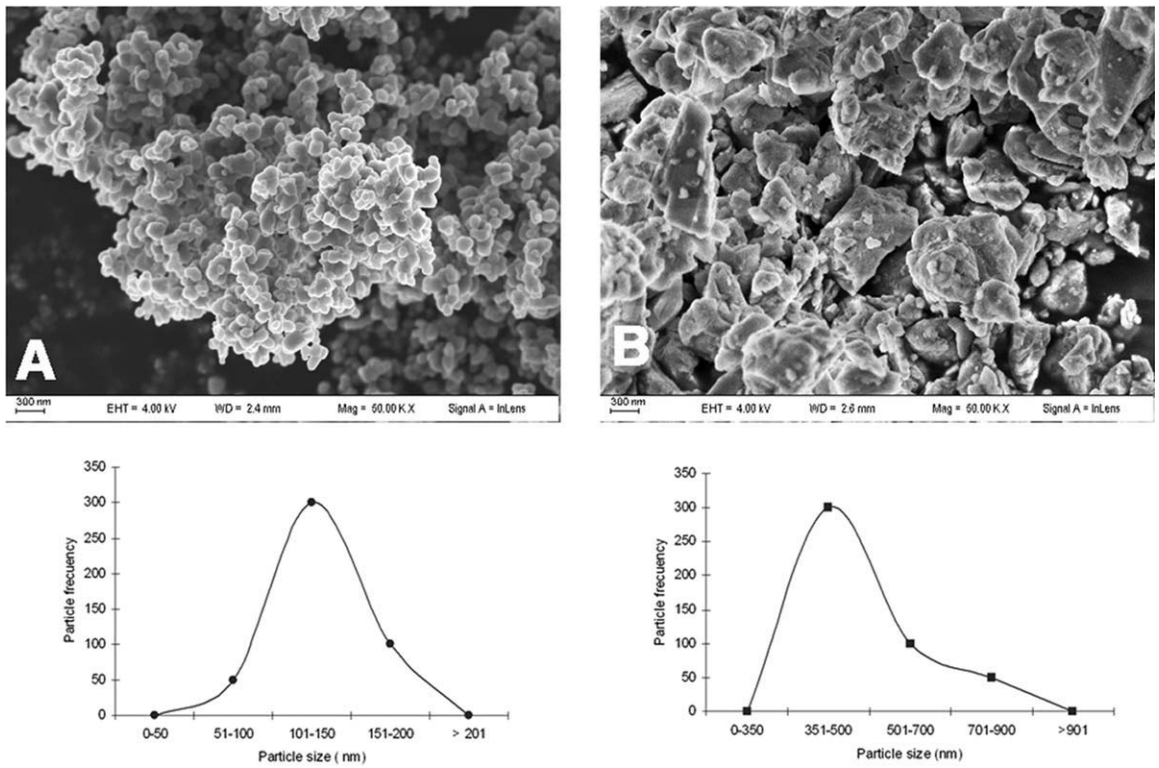

FIGURE 1. Particle morphology and size distribution. SEM microphotographs of TiO_2 (A) and ZrO2 (B) particles. Size distribution is shown in the panels below. ×50,000.

NBT in the presence or absence of TPA, a known inductor of O_2^- generation. BAL suspension obtained from each animal was separated into three fractions as described elsewhere.[22] Briefly, fraction A was used to analyze macrophage percentage in the BAL suspension by differential staining using May–Grunwald–Giemsa. Fraction B was used to evaluate non-TPA stimulated macrophages by adding NBT alone (1 mL, 0.1% in PBS) at 37°C for 60 min under mild agitation to estimate the basal reaction in the cell suspension. Fraction C was used to evaluate TPA-stimulated macrophages; BAL suspension was exposed to NBT alone (1 mL, 0.1% in PBS) for 15 min and then to TPA (10 μL, 100 μg/mL acetone) for another 45 min at 37°C under mild agitation. No less than 3×10^5 cells/mL were incubated for each fraction. All samples were run in duplicates. Cells showing a blue formazan precipitate were considered reactive, whereas those without precipitate were scored as nonreactive. The percentage of reactive and non reactive cells was evaluated by light microscopy.[10]

Oxidative stress markers

To assess the occurrence of oxidative stress, the activity of antioxidant enzymes-SOD, nonenzymatic antioxidants (TRAP), and TBARS (thiobarbituric acid reactive substances), a marker of oxidative damage to lipids, were evaluated.

Preparation of tissue homogenates. Tissue samples (0.2 g of wet weight) were homogenized in 120 mM KCl, 30 m*M* phosphate buffer (pH 7.4) at 0–4°C. The suspension was centrifuged at 600 × *g* at 0–4°C for 10 min to remove

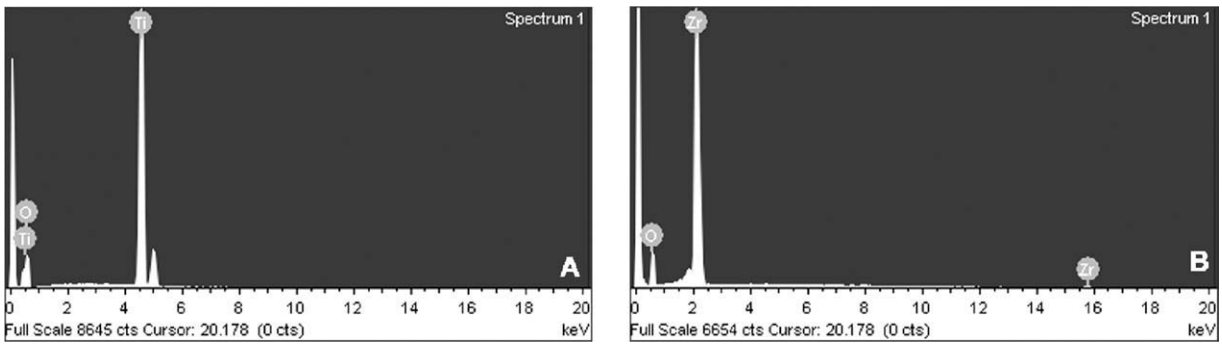

FIGURE 2. EDS showing the presence of titanium (A) and zirconium (B) in the studied particles.

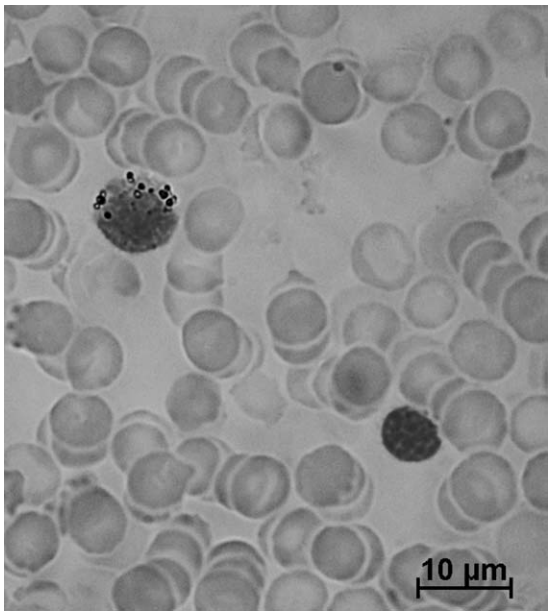

FIGURE 3. Blood smear showing the cytoplasm of a phagocytic mononuclear cell loaded with particles, presumably titanium. Safranin stain. Orig. Mag. ×1000.

nuclei and cell debris. The pellet was discarded, and the supernatant was used as "homogenate."[23]

SOD activity. Superoxide dismutase (SOD) activity was determined spectrophotometrically by measuring inhibition of adenochrome formation rate at 480 nm in a reaction medium containing 1 m*M* epinephrine and 50 m*M* glycine/NaOH (pH 10.5).[24] The enzyme activity was expressed in units/milligram of protein.

Total reactive antioxidant potential. Total reactive antioxidant potential (TRAP) was measured by chemiluminescence. Briefly, the reaction medium used contained 20 m*M* ABAP and 40 µ*M* luminol. ABAP is a source of free radicals that react with luminol yielding chemiluminescence, which was measured in a Packard Tri Carb liquid scintillation counter. The addition of a sample aliquot decreases the chemiluminescence to basal levels for a period proportional to the amount of antioxidants present in the sample, until luminol radicals are regenerated (induction time). The system was calibrated against Trolox, a vitamin E hydrosoluble analogue, and the results were expressed as µ*M* Trolox/mg of protein.[25]

Thiobarbituric acid reactive substances. TBARS was determined using a fluorometric assay.[26] An aliquot of tissue homogenate was added to 2 mL of 0.1*N* HCl, 0.3 mL 10% (w/v) phosphotungstic acid, and 1 mL of 0.7% (w/v) 2-thiobarbituric acid. The mixture was heated in boiling water for 60 min. TBARS were extracted in 5 mL on *n*-butanol. After brief centrifugation, the fluorescence of the butanolic layer was measured in a Perkin Elmer LS 55 luminiscence spectrometer at 515 (excitation) and 553 nm (emission). A calibration curve was prepared using 1,1,3,3-tetramethoxypropane as the standard. Results were expressed as pmol TBARS/mg of protein.

Protein measurement

Protein was measured by the Lowry et al.[27] method using bovine serum albumin as standard.

Statistical analysis

The results were compared using ANOVA or Student's *t* test. Statistical significance was set at $p < 0.05$.

RESULTS

No changes in body weight or behavior were observed in control or treated animals throughout the entire experimental period. No animal death was recorded throughout the experiment.

Morphological and chemical characterization by SEM and EDS

Morphologic analysis by SEM showed differences in shape and average size of Ti and Zr particles (Fig. 1). The Ti particles were spherelike shaped, were evenly distributed, and their average particle size was 0.14 µm ± 0.3 [Fig. 1(A)]. Zr particles were crystal-like shaped, unevenly distributed, and their average particle size was four times greater (0.52 µm ± 0.2) than that of Ti particles [Fig. 1(B)].

Elemental analysis by energy dispersion spectroscopy (EDS) confirmed the presence of Ti and Zr, respectively (Fig. 2).

Histological analysis

The histological study of blood smears corresponding to both Ti and Zr groups showed the presence of mononuclear phagocytic lineage cells loaded with particles at all the experimental time points (Fig. 3). A heterogeneous distribution of cytoplasmic particles was observed. The presence of cells loaded with Ti or Zr particles was observed at all the experimental time-points (3, 6, and 18 months).

Ti and Zr deposits [Fig. 4(A–H)] were observed in liver and lung parenchyma and confirmed by ICP-OES. The particles were observed outside cells and/or phagocytosed by lung alveolar macrophages and liver Kupffer cells. It is noteworthy that, though to a lesser degree, particles were observed in hepatocyte cytoplasm. As shown in Figure 4, Ti and Zr deposits in liver and lung tissue decreased with time. Interestingly, however, Ti deposits [Fig. 4(A,C,E,G)] were larger and more densely compared to Zr deposits [Fig. 4(B,D,F,H)].

Titanium and zirconium concentration in serum, liver, and lung

As shown in Table I, serum Ti and Zr baseline levels (0 months) were <0.6 µg/Lt. Serum control values at 3, 6, and 18 months were similar to baseline values ($p > 0.05$; data not shown). Serum Ti and Zr values decreased significantly with time (3, 6, and 18 months).

Results of the effect of both treatments (Ti and Zr) on lung and liver tissue through time (3, 6, and 18 months)

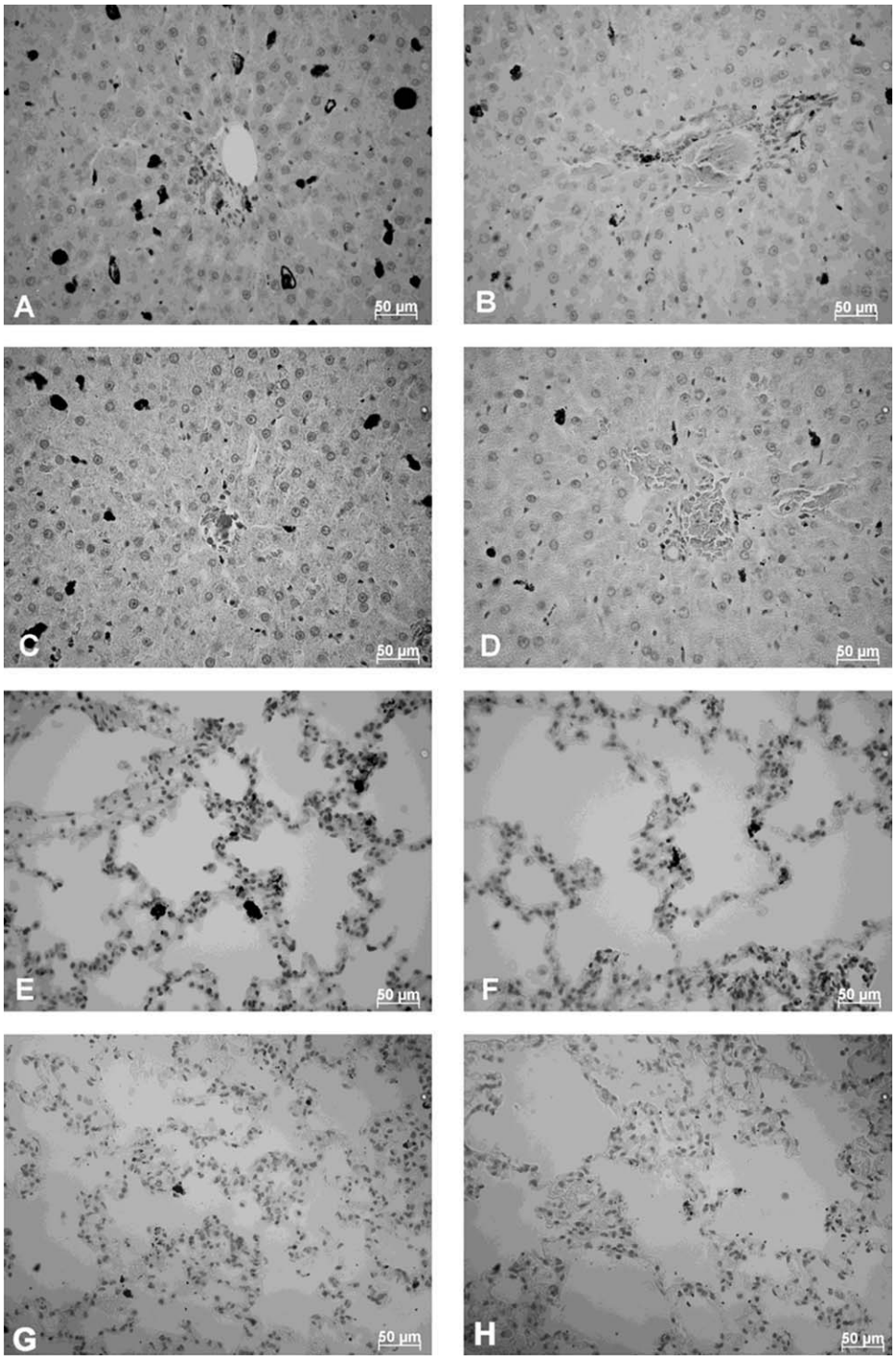

FIGURE 4. Histological analysis of the studied organs. Note the deposits in the liver (A–D) and lung (E–H) parenchyma (Safranin stain), Orig. Mag. ×400. Titanium (A,C,E,G) and zirconium (B,D,F,H) deposits decrease progressively with time. In all cases, titanium deposits are more abundant than zirconium deposits. The figure shows histological samples obtained at 3 and 18 months.

are shown in Table II. Liver and lung baseline levels (0 months) of Ti and Zr were $<$0.8 mg/kg. Both Ti and Zr content decreased significantly in lung tissue through time compared to controls ($p < 0.05$). Unlike Zr content, Ti content was also found to decrease significantly in liver tissue. It is noteworthy that Zr content in lung tissue was 5–10 times lower than Ti content. However, the same was not observed in liver tissue. Control values corresponding to lung and liver tissue at 3, 6, and 18 months did not differ significantly from baseline values ($p > 0.05$; data not shown). These results are consistent with the aforementioned histologic findings.

TABLE I. Quantitative Evaluation of Ti and Zr Content in Serum

	Serum (μg/Lt)	
Months	Ti	Zr
0	<0.6	<0.6
3	66.9 ± 27.7	46.3 ± 7.6
6	*10.5 ± 3.2 *	*16.6 ± 1.9*
18	<8.0	19.7 ± 5.8

Serum values of Ti and Zr decreased significantly through time (between 3 and 18 months). Values are mean ± SD;
*$p < 0.05$.

Generation of superoxide anion (O_2^-) in alveolar macrophages

Light microscopy observations of BAL cells obtained from control and treated animals revealed that the generation of ROS varies among the macrophage population. Figure 5 shows the presence of a nonreactive cell and a highly NBT reactive macrophage in BAL of a TiO_2-treated animal.

Figure 6 shows that the generation of O_2^- in BAL from lung tissue of Ti-treated animals rose significantly through time compared to the Zr and C groups. No differences in superoxide anion levels were found between Zr-treated and control animals at 3 and 6 months. Only at the longest experimental time did Zr cause a significant increase in O_2^- generation compared to the C group. Nevertheless, this increment was lower than that elicited by Ti particles (Co: 29.62 ± 3.9; Zr: 3.9 ± 4.7; Ti: 51.1 ± 5.9; $p < 0.05$).

Evaluation of antioxidants (SOD and TRAP) and thiobarbituric acid reactive substances

SOD activity in the lung of Ti-treated animals decreased significantly at all experimental time points. However, SOD activity in liver decreased only at 6 and 18 months ($p < 0.05$; Fig. 7(A,D)]. Interestingly, the decrease was consistently greater in Ti than in Zr-treated animals. Nonenzymatic antioxidant level (TRAP) only decreased significantly in Ti exposed lung tissue at the longest experimental time point ($p < 0.05$; Fig. 7(B,E)]. Examination of membrane damage (TBARS) showed no changes in lung or liver tissue through time [Fig. 7(C,F)].

DISCUSSION

Particles can enter the body through inhalation, ingestion, or skin absorption.[28] In addition, particles might also be found in the body as a result of their release from the metallic implant surface of biomedical devices,[18,29] due to electrochemical dissolution, frictional wear, or a synergistic combination of the two.[15] To our knowledge, the chemical forms of these released elements have not been identified. It is unclear whether these products remain as metal ions or metal oxides or if they form protein or cell-bound complexes.[30,31] In the particular case of titanium, little is known about the valence with which it exerts its action, the organic or inorganic nature of its ligands, and its potential toxicity.[32]

The *in situ* degradation of a metal implant is detrimental, because it not only alters the structural integrity of the implant but also causes metal particles/ions to be released, remaining in the peri-implant milieu[6,33] and/or migrating systemically.[10,34–37] Numerous reports in the literature describe histological evidence of inflammatory response and the presence of metallic particles varying in shape and size in the tissues adjacent to orthopedic prostheses of titanium or titanium-based alloys.[15] Likewise, previous works performed at our laboratory showed the presence of titanium particles in tissues adjacent to failed human dental implants[16] and in reactive lesions of the peri-implant mucosa[38]. According to the literature, the size of particles stemming from the surface of an implant and found in body tissues ranges from nanometers to millimeters. In view of this, particles equivalent in size to those observed histologically in tissues were used in the present study.

Although it holds true that the experimental doses used herein are high in terms of a normal *in vivo* situation, they served the purpose of this study, because they allow rapid observation of the adverse effects of particles in the studied tissue.[17]

"Physicochemical properties that may be important in understanding the toxic effects of test materials include particle size and distribution, agglomeration state, shape, crystal structure, chemical composition, surface area, surface chemistry, surface charge, and porosity."[39]

As previously stated, the degree of cytotoxicity correlates to particle size. In the present study, average size of TiO_2 particles was smaller than that of ZrO_2 particles. According to our results, particle size would seem to have had an effect on biological response. At equal doses of injected particles, superoxide anion generation in alveolar macrophages was higher in TiO_2 than in ZrO_2-treated animals. Our results are in agreement with a number of studies showing that smaller particles are more toxic than larger ones.[40–42] Oberdörster

TABLE II. Quantitative Evaluation of Zr and Ti Content in Lung and Liver

	Lung (mg/kg)		Liver (mg/kg)	
Months	Zr	Ti	Zr	Ti
0	<0.8	<0.8	<0.8	<0.8
3	10.3 ± 3.3*	91.3 ± 15.4*	ND	684 ± 151.3*
6	*6.2 ± 3.2	*38.7 ± 17.6	252.9 ± 62.1	*430 ± 70
18	4.6 ± 3.3	28.5 ± 12.1	216.2 ± 21.1	217.1 ± 65.6

Zr and Ti content decreased significantly in lung tissue with time. Only Ti deposits decreased significantly in liver tissue. Values are mean ± SD;
*$p < 0.05$. ND, not determined.

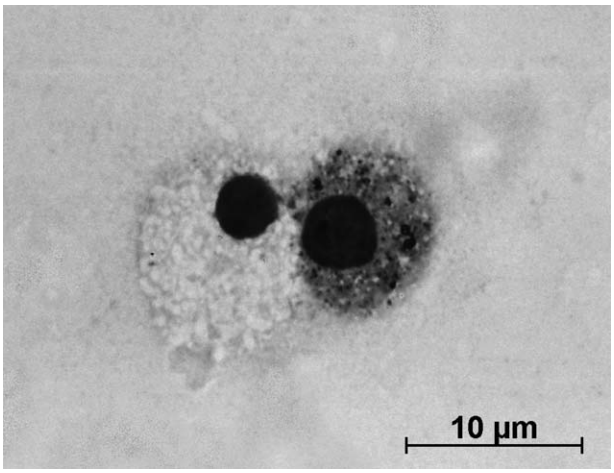

FIGURE 5. Photomicrograph showing the presence of a nonreactive and a highly NBT reactive macrophage in BAL of a TiO_2-treated animal. Note intense NBT staining on a TiO_2-loaded macrophage. The stronger the blue stain, the higher the rate of superoxide anion generation.

et al.[28,39] showed that when the same gravimetric doses of ultrafine and fine TiO_2 particles were delivered to the lung, ultrafine particles produced significantly greater inflammation and interstitial translocation, lending support to the idea that the smaller the particle, the greater the injury.

It must be pointed out that ZrO_2 has a distribution skewed to the right (Fig. 1), implicating that a larger mass of injected particles is represented by larger particles, conceivably reducing systemic translocation. It is therefore possible that this characteristic of zirconium particles may account for the smaller size of these deposits when compared with the titanium deposits.

Although our data are limited, other studies have also demonstrated the importance of "surface area" in the adverse biological effect of both fibrous and spherical particles.[43] Toxicity induced by particles correlates not only to particle size but also particle shape.[44] Particle shape may also have effects on the kinetics of deposition and absorption in the body.[39] The shape of the particles used in the present study (sphere-like shaped TiO_2 particles and crystal-like shaped ZrO_2 particles) may also have affected biological response.

Apart from size and shape, other structural characteristics may also be involved in the various cytotoxic effects. Warheit et al.[45] demonstrated that crystal structure and surface chemical reactivity may also affect biological response. In particular, chemical reactive groups present at the surface of the particles may differ from one particle type to another, therefore leading to various cytotoxic profiles when interacting with different cell types.[39] The differences in biological response observed in this study may be associated with the crystal structure of the particles used: anatase in the case of titanium and baddeleyite in the case of zirconium.

Macrophages are cells that respond to *in vivo* implantation of biomaterials depending on the size and structure of the material, among other variables.[46] Particles that are smaller than the macrophages themselves (<10 µm) can be easily phagocytosed.[47] Moreover, crystal structure may also influence either the tendency for a particle to be internalized by phagocytes or the subsequent cell response.[48]

Our results showed plasma concentration of Zr and Ti to decrease significantly through time. However, results obtained with blood smears showed the presence of cells of the mononuclear-phagocytic lineage loaded with particles at all the experimental time points. In addition, particle deposits in body organs decreased between 3 and 18 months, indicating the occurrence of clearance. Given that neither plasma concentrations of Zr or Ti decreased between months 6 and 18 but reached a plateau, it is possible that the particles resulting from the clearance entered the plasma complexed to proteins or, as observed herein, phagocytosed by cells of the mononuclear phagocytic system.

In the present study, differences in oxidative metabolism were observed between alveolar macrophages exposed to Zr and those exposed to Ti. Titanium was found to induce higher O_2^- generation at all the experimental time points, whereas Zr caused an increase in oxidative metabolism at 18 months only, inducing no changes at 3 and 6 months. It is noteworthy that O_2^- generation was consistently higher in Ti-exposed animals compared to those exposed to Zr. Likewise, SOD and TRAP consumption in lung tissue were greater in Ti-treated animals. This decrease could be correlated to the increase in superoxide production. As regards liver tissue, SOD (antioxidant enzyme) consumption was higher than TRAP (non–antioxidant enzymes) at all the studied time points. No significant changes in TBARS were observed in either lung or liver tissue of either group through time. Thus, we suggest that the oxidative stress condition does not affect membrane phospholipids due to the compensatory action of the antioxidants in the tissue.

Clearance was always higher in Ti-treated than in Zr-treated animals; however, it must be kept in mind that the quantity of Ti deposited in organs and O_2^- generation in Ti exposed lung tissue were consistently higher when compared with Zr. This behavior suggests better biological response to Zr than to Ti.

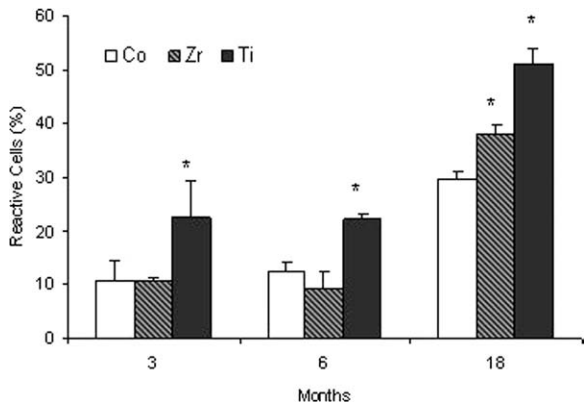

FIGURE 6. Effect of Ti or Zr on superoxide anion release by BAL cells through time. The percentage of reactive cells was determined by the NBT reduction test. Values are mean ± SD; $*p < 0.05$.

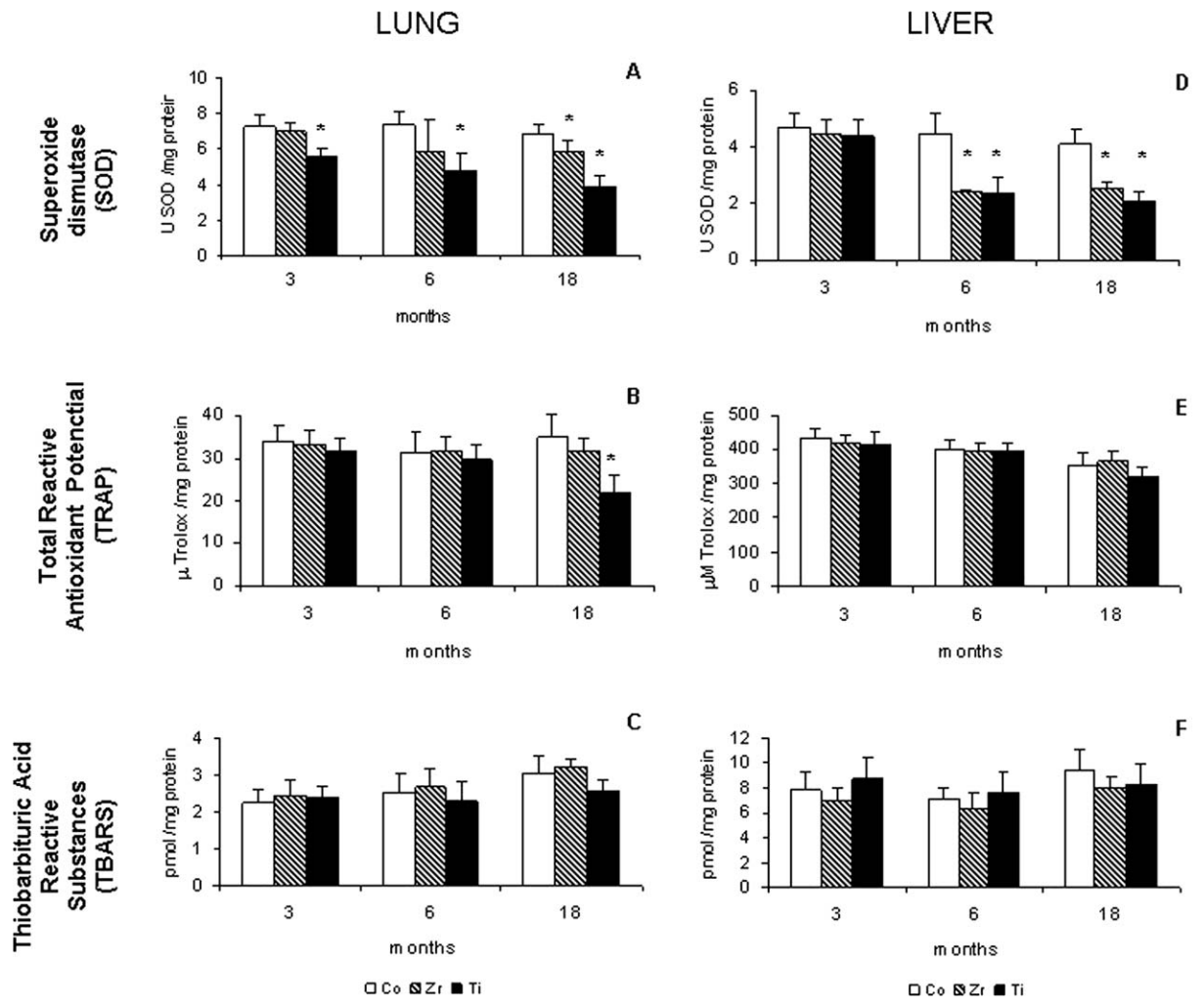

FIGURE 7. Lung and liver superoxide dismutase (SOD), total antioxidant reactive potential (TRAP), and thiobarbituric acid reactive substances (TBARS) levels for control and Ti or Zr-treated animals through time. Values are mean ± SD; $*p < 0.05$.

From the point of view of implantology, Thomsen et al.[2] found that both Ti and Zr have a positive effect on tissue-material interaction. A previous experimental study conducted at our laboratory on bone tissue response to an implant showed greater peri-implant bone thickness and volume in bone surrounding Zr implants when compared with that around Ti implants.[49]

Zirconium is chemically related to and has several properties in common with titanium. According to several researchers,[2,3,50,51] the elasticity, corrosion resistance, and other physicomechanical properties of Zr and its alloys make them a suitable material for implants used in medicine. Because Zr offers superior corrosion resistance over most other alloy systems, better behavior in biological environments can be presumed.[52] Nevertheless, it is not widely used as a clinical material at present,[2] because commercial manufacture of implants from zirconium or its alloys seems to be unfeasible due to the high cost of this material.[3]

The potential uses of Zr-based materials for prosthetics and dental applications should be strongly considered and further investigated in laboratory and clinical settings.

In vivo studies of the biological effects of substances such as metal ions, chemicals, micro, and nanoparticulate matter require knowledge about their distribution, target organs, destination, and potential risk. Although both titanium and zirconium are transition metals, their physicochemical properties, such as oxidation velocity, interaction with water, crystalline structure, and transport properties, and/or those of their oxides differ quantitatively. In this regard, the results of the present study show that the biokinetics of TiO_2 and ZrO_2 microparticles depends on differences in physicochemical properties of the particles, such as size, shape, and/or crystal structure.

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