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# Impact through time of different sized titanium dioxide particles on biochemical and histopathological parameters

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Received 22 March 2013; revised 21 May 2013; accepted 31 May 2013
Published online in Wiley Online Library (wileyonlinelibrary.com). DOI: 10.1002/jbm.a.34822

**Abstract:** Due to corrosion, a titanium implant surface can be a potential source for the release of micro (MPs) and nano-sized particles (NPs) into the biological environment. This work sought to evaluate the biokinetics of different sized titanium dioxide particles ($TiO_2$) and their potential to cause cell damage. Wistar rats were intraperitoneally injected with 150 nm, 10 nm, or 5nm $TiO_2$ particles. The presence of $TiO_2$ particles was evaluated in histologic sections of the liver, lung, and kidney and in blood cells at 3 and 12 months. Ultrastructural analysis of liver and lung tissue was performed by TEM, deposit concentration in tissues was determined spectroscopically, and oxidative metabolism was assessed by determining oxidative membrane damage, generation of superoxide anion ($O_2^-$), and enzymatic and non-enzymatic antioxidants. $TiO_2$ particles were observed inside mononuclear blood cells and in organ parenchyma at 3 and 12 months. $TiO_2$ deposits were consistently larger in liver than in lung tissue. Alveolar macrophage $O_2^-$ generation and average particle size correlated negatively ($p < 0.05$). NPs were more reactive and biopersistent in lung tissue than MPs. Antioxidant activity, particularly in the case of 5 nm particles, failed to compensate for membrane damage in liver cells; the damage was consistent with histological evidence of necrosis. © 2013 Wiley Periodicals, Inc. J Biomed Mater Res Part A: 00A:000–000, 2013.

**Key Words:** titanium dioxide, microparticles, nanoparticles, biokinectics, macrophages

**How to cite this article:** Bruno ME, Tasat DR, Ramos E, Paparella ML, Evelson P, Rebagliati RJ, Cabrini RL, Guglielmotti MB, Olmedo DG. 2013. Impact through time of different sized titanium dioxide particles on biochemical and histopathological parameters. J Biomed Mater Res Part A 2013;00A:000–000.

## INTRODUCTION

The population at large is exposed to different types of particles, which can enter the body through the skin, the respiratory system, or the gastrointestinal tract.[1] As a result of the widespread use of metallic biomaterials in areas such as orthopedics and dentistry, the surface of biomedical devices could be a potential source of systemic contamination due to the release of particles.[2–4] Titanium (Ti) is the most commonly used biomaterial in the manufacture of biomedical implants.[5] It is highly reactive, and on exposure to air or fluids it rapidly develops a layer of titanium dioxide ($TiO_2$) that passivates the metal. This layer is responsible for biocompatibility and constitutes the interface between the biological milieu and the implant, decreasing its reactivity and partly preventing corrosion.[2,6,7] Nevertheless, no metal or metal alloy is completely inert *in vivo*. Because metallic implants are in contact with body tissue and fluids, ions/particles could be released into the biological milieu as a result of electrochemical processes.[2] It must be pointed out that ions/particles may be released from metal implants as a result of electrochemical corrosion processes, frictional wear, or a synergistic combination of the two.[2] It is well documented that released particles/ions can trigger different biological effects.[2,8–13]

Histological findings of previous experimental studies conducted at our laboratory demonstrated the presence of particles in the peri-implant tissues surrounding titanium implants.[14,15] In agreement with reports in the literature,[8,9,16–18] our studies also demonstrated the presence of titanium particles in peri-implant tissue from failed human dental implants,[19] in oral mucosa associated with implant

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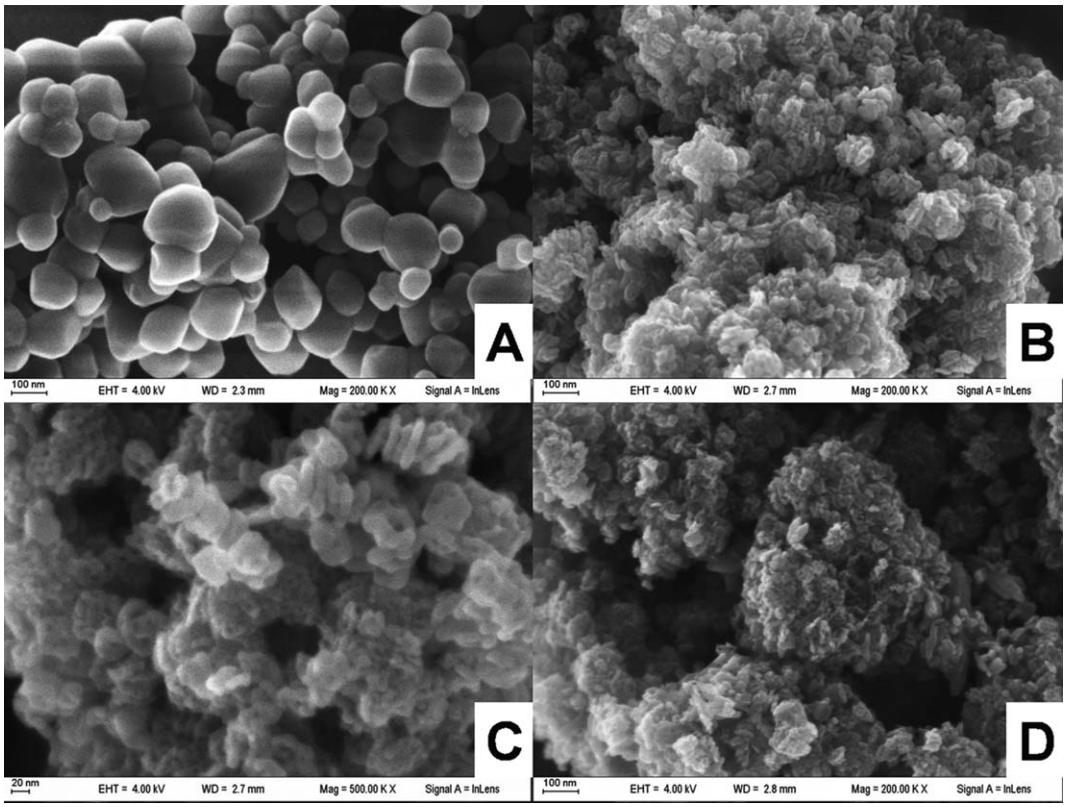


**FIGURE 1**. SEM images of $TiO_2$. Note the different sizes and shapes: (A) 150nm, (B and C) 10nm, (D) 5nm. (A, B, and D) (X200.000) – (C) (X500.000).

cover screws,[20] in cells exfoliated from peri-implant oral mucosa around titanium dental implants,[21] and in reactive lesions of peri-implant mucosa.[22] At the systemic level, our experimental studies showed that titanium particles are transported in the blood via cells of the mononuclear phagocytic lineage[23] and are deposited in organs with macrophagic activity such as the liver, spleen, and lungs,[24–27] causing an increase in oxidative stress in lung macrophages.[28,29]

The surface of a metallic medical implant may be a potential source of release not only of microparticles (MPs) (>100 nm), but also of nanoparticles (NPs) (1–100 nm) into the biological milieu. Whereas the biological effects of titanium MPs released from the implant surface are well documented, little is known about the biological effects, biodistribution, and final destination in the body, of titanium NPs. Given that NPs have a larger surface to volume ratio, they are biologically more reactive and potentially more harmful to human health than MPs. Although MPs and NPs can be chemically similar, their specific physical–chemical properties may cause different biological responses.[4] As a rule, metal implants used in orthopedics and/or dentistry remain in place over long periods of time. It is thus important to study and define the chemical features of the released particles, the quantity of particles that enter the biological milieu and their biopersistence, the sites where the particles are transported, and their physio-pathological consequences through time.

Understanding the biological effects of different sized particles on tissues surrounding dental or orthopedic implants and on body systems is a new challenge to nanotoxicology and biocompatibility studies.

In view of the above, the aim of the present study was to evaluate the biokinetics (distribution, destination, and deposition) and potential health risks of different sized titanium particles that could originate from the surface of biomedical implants.

## MATERIALS AND METHODS

### Animal treatment protocol

Following our experimental model,[24] male Wistar rats ($n = 60$) weighing ~100 g were injected intraperitoneally (i.p.) with a suspension of $TiO_2$ particles in 5 mL 0.9%

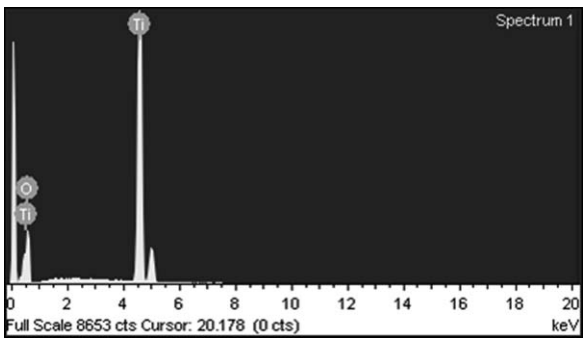


**FIGURE 2**. EDS spectrum with the peak corresponding to titanium in the studied particles.

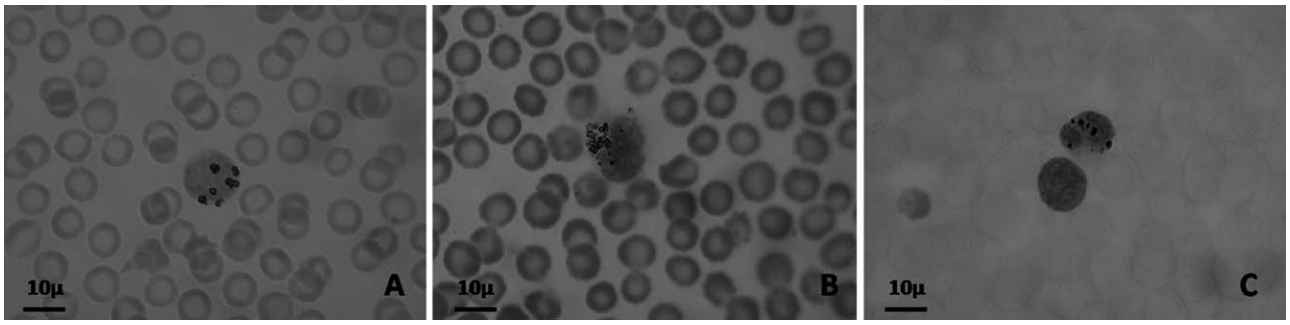


**FIGURE 3**. Microphotographs of monocytes-containing intracytoplasmic particles. (A) $TiO_2$-MP150 group, (B) $TiO_2$-NP10 group, and (C) $TiO_2$ -NP5 group. Safranin Stain. Orig. Mag. X1000.

ClNa, at a dose of 1.60 g/100g body weight. The experimental treatments were as follows: $TiO_2$-MP150 ($n = 20$): i.p. injection with $TiO_2$ (anatase), average particle size (APS): 150nm; Sigma Chemical Company; $TiO_2$-NP10 ($n = 20$): i.p. injection with $TiO_2$ (anatase), APS: 10nm - Nanostructured and Amorphous Materials, Los Alamos, NM; and $TiO_2$-NP5 ($n = 20$): i.p. injection with $TiO_2$ (anatase), APS: 5nm - Nanostructured and Amorphous Materials, Los Alamos, NM. Control animals ($n = 20$) were injected with an equivalent volume of vehicle.

Baseline titanium levels in plasma and organs were determined as explained in point "Quantitation of baseline titanium levels in plasma and organs".

The animals were euthanized at 3 or 12 months post injection. Blood samples were obtained by cardiac puncture prior to euthanasia. Immediately following euthanasia, alveolar macrophages (AM) were obtained by bronchoalveolar lavage (BAL), and the lungs, liver, and kidneys were excised.

All procedures were performed in compliance with the National Institutes of Health (NIH) guidelines for the care and use of laboratory animals [NIH Publication N° (85-23) Rev. 1985] and the guidelines of the School of Dentistry of the University of Buenos Aires [Res. (CD) 352/02 and Res. (CD) 694/02].

**Particle characterization: Scanning electron microscopy (SEM) and energy-dispersive X-ray spectroscopy (EDS)**

APS was confirmed by SEM using a Supra 40 Zeiss microscope equipped with a field emission filament. The particles were placed on a conductive carbon tape and analyzed without being coated. Images were obtained using an in-lens detector and 4 kV acceleration voltage. Particles were chemically identified by EDS using an Oxford Instrument detector.

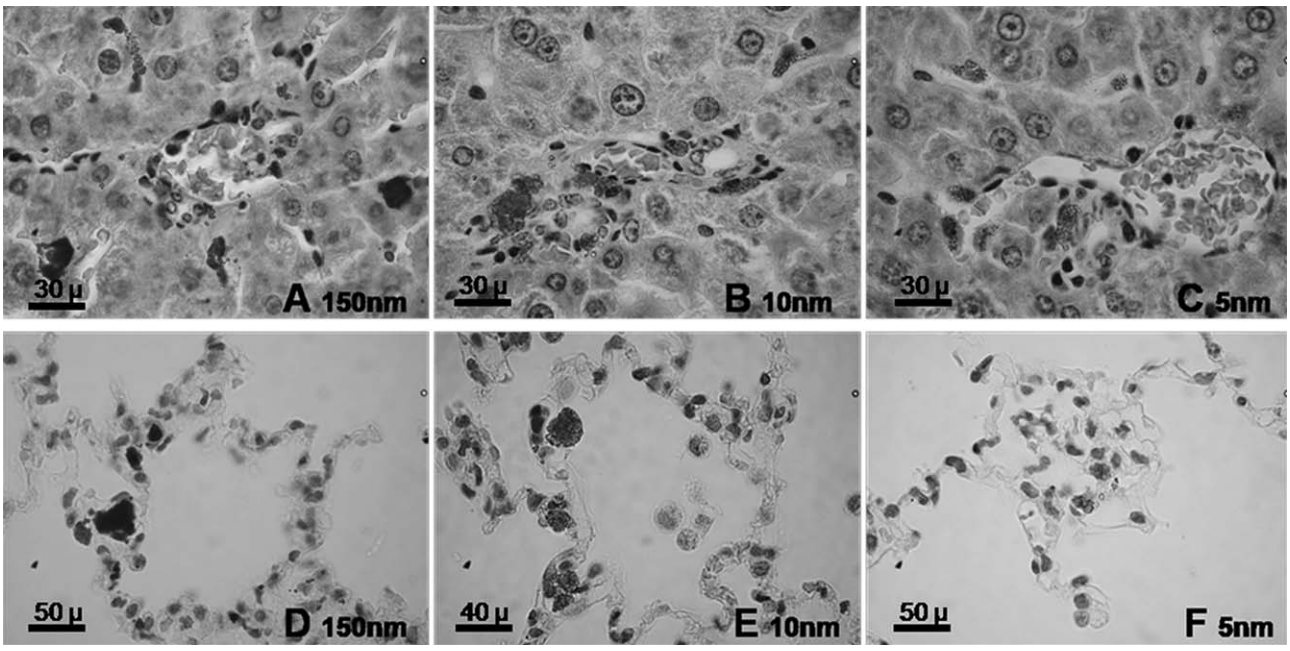


**FIGURE 4**. (A–F) Microphotographs showing the presence of $TiO_2$ particles in paraffin embedded sections at 3 months post-injection. (A–C) Liver. Ti deposits increased with average particle size (APS). H-E Stain. Orig. Mag. X1000; (D–F) Lung. The density of the Ti deposits correlated directly with APS. Safranin Stain. Orig. Mag. X1000.

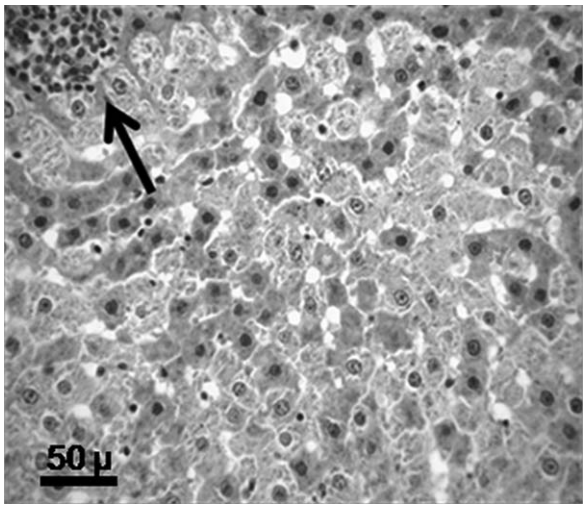


**FIGURE 5**. Liver (3 months). Areas of necrosis with focalized chronic inflammatory infiltrate (→) and extravasated erythrocytes can be observed. $TiO_2$-NP5 Group. H-E Stain. Orig. Mag. X400.

### Quantitation of baseline titanium levels in plasma and organs

Prior to injecting the titanium oxide solution or vehicle, a blood sample (2 $cm^3$) was obtained from each animal by cardiac puncture in order to quantitate baseline plasma levels of titanium. In addition, the liver and lungs were excised from an additional group of five animals for determination of baseline levels of titanium in both organs. All determinations were performed using inductively coupled plasma mass spectroscopy (ICP-OES; Perkin Elmer Optima 3100).

### Quantitation of post-injection titanium levels in plasma and organs

Plasma and organ levels of titanium were determined in blood and in liver and lung samples obtained at 3 and 12 months, by ICP-OES analysis.

### Histological analysis (light and transmission electron microscopy)

Samples of liver, lung, and kidney tissue obtained at 3 and 12 months post-injection were fixed in 10% formalin and embedded in paraffin; sections were obtained and stained with H–E and Grenacher's carmine for histological examination under a light microscope. The sections were treated with a saturated picric acid solution to remove formalin pigment. In addition, samples of liver and lung tissue were processed for ultrastructural observation by transmission electron microscopy (TEM, Electron Microscope Zeiss EM 109T). The presence of titanium in blood cellular components was determined on blood smears stained with safranin, which facilitates visualization of phagocytosed matter.

### Analysis of oxidative metabolism

***Determination of superoxide anion ($O_2^-$) in alveolar macrophages.*** Superoxide anion ($O_2^-$), a main reactive oxygen specie generated during the respiratory burst, was evaluated in AM using the Nitro Blue Tetrazolium (NBT) reduction test.[30] AM were recovered from control and experimental animals by BAL, as described elsewhere.[31] Cells showing a blue formazan precipitate were considered reactive, whereas those without precipitate were scored as non reactive. Reactive and non reactive cells were counted using light microscopy, and the percentage of each was calculated.[32]

***Determination of oxidative damage to lipids.*** Oxidative damage to lipids was determined by measuring thiobarbituric acid reactive substances (TBARS) levels using a fluorometric assay;[29] 0.5 mL of tissue homogenate were added to a medium consisting of 0.1 *N* HCl, 10% (w/v) phosphotungstic acid and 0.7% (w/v) 2-thiobarbituric acid. After incubation in boiling water for 60 min, TBARS were extracted with *n*-butanol. The fluorescence of the butanolic layer was measured in a Perkin Elmer LS 55 flourometer at 515 nm (excitation) and 553 nm (emission). A calibration curve was performed using 1,1,3,3-tetramethoxypropane as standard. Results were expressed as pmol TBARS/mg protein.

***Determination of enzymatic (SOD, CAT) and hydrosoluble non-enzymatic (TRAP) antioxidants.*** Antioxidant species were evaluated in liver and lung homogenates at 3 months post-injection only, following the method described by

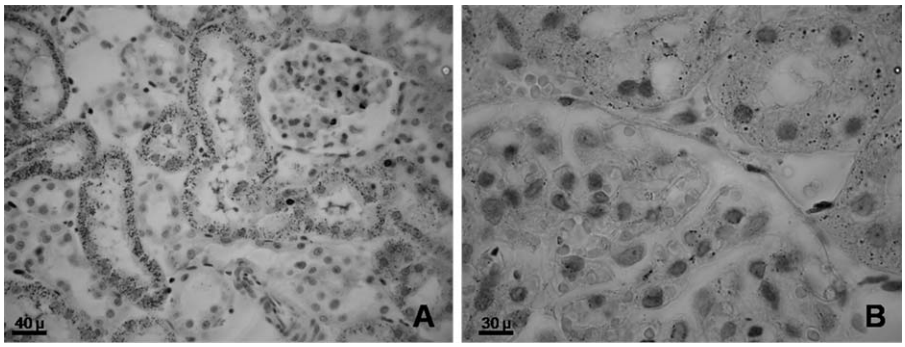


**FIGURE 6**. Kidney (12 months). (A) $TiO_2$-NP10 deposits inside cells from the convoluted tubules. Safranin Stain. Orig. Mag. X400; (B) Deposits can be seen in more detail at higher magnification. Orig. Mag. X1000.

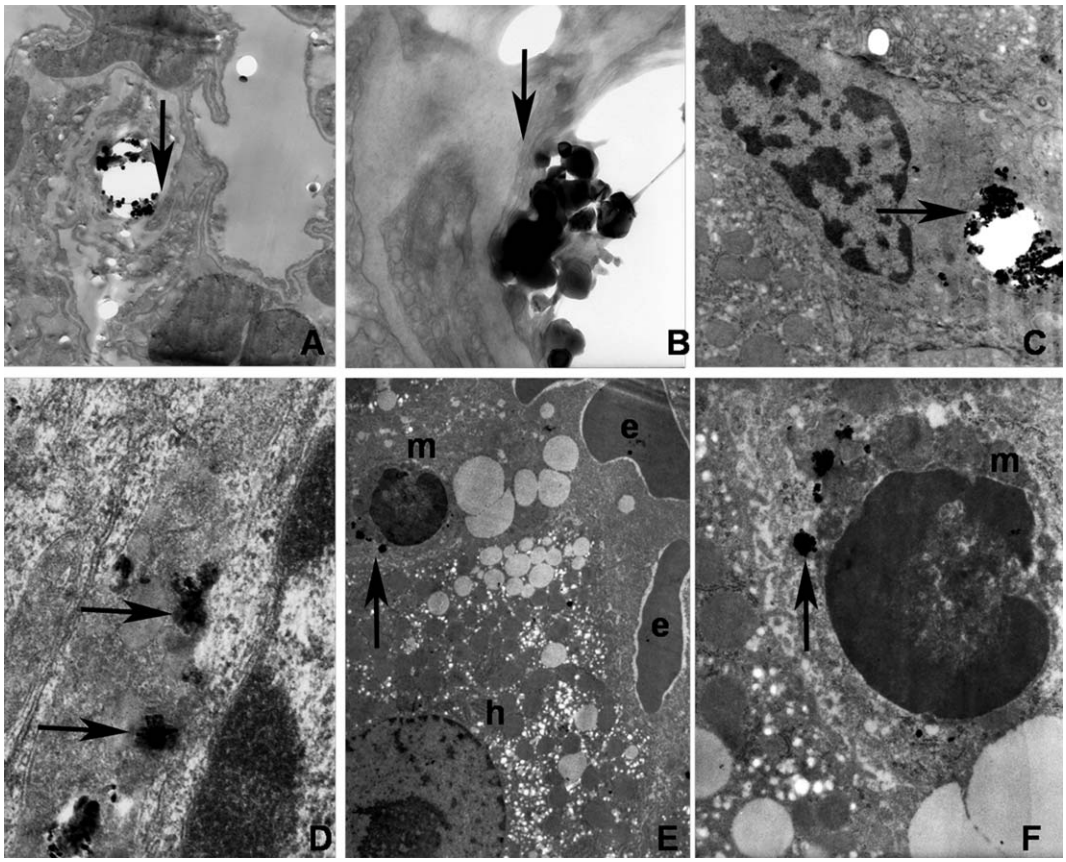


**FIGURE 7**. (A–F) Localization of $TiO_2$ particles in ultrathin sections (TEM). (A and B) $TiO_2$-MP150 in lung parenchyma. (A) A pneumocyte with particles inside a vesicle (→). X7000 and (B) Numerous membrane-bound aggregates (→) can be observed at higher magnification. X50000. (C and D) $TiO_2$-NP10 in the liver. (C) Particles contained in a vesicle (→) of a Kupffer cell X10.000. (D) Particle aggregates (→) close to the nucleus of another Kupffer cell can be seen at greater magnification. X31.500. (E and F) $TiO_2$-NP5 in a hepatic sinusoidal cell. (E) A monocyte containing an aggregation of $TiO_2$ particles (→) close to the nucleus. X4000. (m) monocyte, (h) hepatocyte, (e) erythrocyte. (F) Multiple NPs (→) inside the cell can be observed at higher magnification X26000.

LLesuy et al.[33] *Superoxide dismutase (SOD)* activity was determined following the methodology employed by Misra and Fridovich.[34] Enzymatic activity is expressed as SOD units/mg protein. *Catalase (CAT)* activity was evaluated as described by Chance B.[35] Results are expressed as pmol catalase/mg protein. *Total reactive antioxidant potential (TRAP)* was measured by a chemiluminescent method.[36] Results are expressed as μM Trolox/mg protein.

### Protein content

Protein concentration was measured using bovine serum albumin as standard, following the method described by Lowry et al.[37]

### Statistical analysis

The results were compared using One-Way ANOVA and Newman–Keuls or Bonferroni post-hoc test, accordingly. Statistical significance was set at $p < 0.05$.

## RESULTS

No changes in body weight, behavior, or general health were observed in any of the experimental or control animals.

### Particle characterization

Morphological characterization by SEM confirmed APS to be 150, 10, and 5 nm (Fig. 1). The 150 nm particles were mostly spherical [Fig. 1(A)], whereas the smaller particles were lentil-shaped and appeared in clusters [Fig. 1(B–D)]. Chemical analysis by EDS confirmed the presence of titanium in all the particles (Fig. 2).

### Histological analysis

***Light microscopy.*** The histological study of blood smears revealed the presence of $TiO_2$ particles in the cytoplasm of mononuclear phagocytic cells (monocytes) in all the experimental groups at both the studied time points (3 and 12 months) (Fig. 3). It must be pointed out that the particles followed different distribution patterns within the cell. No particles were found in control monocytes at either studied time point.

Histological evaluation of liver and lung sections showed deposits of $TiO_2$ particles in the parenchyma of both organs, either outside the cells or phagocytosed by AM in the lung or by Kupffer cells in the liver. The number and size of the deposits increased with APS in both tissues, at both experimental time points.

**TABLE I. Determination of Ti Particle Concentration in Plasma (ICP-OES)**

| APS (nm) / months | MP 150 | NP 10 | NP 5 |
|---|---|---|---|
| 0 | < 0.8 | < 0.8 | < 0.8 |
| 3 | * < 0.8 | * < 0.8 | * < 0.8 |
| 12 | 11.56 ± 3.62 | 9.25 ± 1.3 | 12.8 ± 3.16 |

Particle concentration is expressed in mg/L as mean ± standard deviation (*$p < 0.05$). APS, average particle size; nm, nanometers.

Microphotographs of tissue sections obtained at 3 months are shown for the purpose of illustration [Fig. 4(A–F)].

Foci of necrosis were observed in liver sections of $TiO_2$-NP10 and $TiO_2$-NP5 treated animals, at 3 and 12 months. Interestingly, a hemorrhagic exudate with infiltration of mononuclear cells and polymorphous nuclear neutrophils was observed in the $TiO_2$-NP5 group. A microphotograph of a liver section obtained 3 months post-injection is shown for illustration (Fig. 5). No morphological changes were observed in lung tissue sections in either of the experimental groups at either time point. As regards the kidneys, particle deposits were observed in sections obtained at 12 months only, and were located in the proximal and distal convoluted tubules and in glomeruli. It is of note that particle deposits were observed in all the experimental groups. A microphotograph of a sample corresponding to the $TiO_2$-NP10 group is shown to exemplify particle deposition (Fig. 6).

***Transmission* electron microscopy.** Ultrastructural analysis of liver and lung tissue using TEM showed MPs and NPs located within vesicles. This observation is indicative of phagocytic internalization of $TiO_2$ particles [Fig. 7(A–F)].

**Quantitation of titanium levels in plasma and body organs**

Plasma concentration of $TiO_2$ changed with time, showing a significant increase at 12 months ($p < 0.05$) in all the experimental groups (Table I).

$TiO_2$ concentration differed in lung and liver. The baseline concentration of $TiO_2$ particles in both organs was $< 0.8$ mg/Kg, and did not vary significantly in control animals at either 3 or 12 months ($p > 0.05$) (data not shown). As shown in Table II, $TiO_2$ concentration in lung tissue decreased with APS at both experimental time points. The decrease in $TiO_2$ concentration reached statistical significance ($p < 0.05$) when comparing lung samples of $TiO_2$-MP150 and NP- treated animals obtained at 3 months. The differences at 12 months post-injection only reached statistical significance between the groups injected with the largest and smallest particles ($p < 0.05$). It is of note that $TiO_2$ concentration in lung tissue decreased significantly with time in the case of MP150 treated animals, but not in the NP10 and NP5 groups ($p > 0.05$) (Table II).

As regards liver samples, APS did not affect $TiO_2$ particle concentration. Nevertheless, a significant time-dependent decrease in $TiO_2$ concentration was observed in liver samples of NP10 treated animals (Table III).

**TABLE II. Determination of $TiO_2$ (ICP-OES) in Lung Tissue**

| APS (nm) / months | MP 150 | NP 10 | NP 5 |
|---|---|---|---|
| 3 | # 35.2 ± 8.10 | 11.8 ± 1.70 * | 5.2 ± 2.60 * |
| 12 | 14.8 ± 7.14 | 6.83 ± 2.71 * | 4.62 ± 1.47 * |

$TiO_2$ concentration decreased with APS (mean ± SD, *$p < 0.05$). Only MP150 decreased significantly through time (mean ± SD, #$p < 0.05$). Particle concentration is expressed in mg/kg. APS, average particle size; nm, nanometers.

**TABLE III. Determination of $TiO_2$ (ICP-OES) in Liver Tissue**

| APS (nm) / months | MP 150 | NP 10 | NP 5 |
|---|---|---|---|
| 3 | 291.6 ± 43.4 | 284.2 ± 46.2 | 263.9 ± 48.9 |
| 12 | 312 ± 66.6 | * 174.25 ± 88.6 | 236.75 ± 37.3 |

$TiO_2$ concentration decreased significantly through time in the NP10 group only (mean ± SD, $*p < 0.05$). Particle concentration is expressed in mg/kg. APS, average particle size; nm, nanometers.

### Analysis of oxidative metabolism

***Determination of superoxide anion ($O_2^-$).*** As can be seen in the microphotograph shown in Figure 8(A), AM loaded with $TiO_2$ particles showed an intense reaction to the NBT test (dark blue-violet color) as a result of formazan precipitate, in all the experimental groups. Superoxide anion generation by AM in MP and NP groups was significantly different compared to controls at 3 and 12 months. The increase in oxygen reactive species correlated inversely with APS, and significant differences were observed between $TiO_2$-NP5 and MP-150 treated animals at both experimental time points [Fig. 8(B,C)].

***Determination of oxidative damage to lipids (TBARS).*** Oxidative damage to lipids, as measured by TBARS, showed no significant differences in membrane damage among the experimental lung samples. For example, the values of $TiO_2$-NP5 animals compared to controls were 1342 ± 382 vs. 1818 ± 555 pmol/ mg protein, respectively.

Although an increase in TBARS content was observed in liver samples from all the experimental groups, the increase was only significant ($p < 0.05$) between the $TiO_2$-NP5 and control groups ($TiO_2$-NP5: 178 ± 29 vs. Co:133 ± 36 pmol/mg protein).

***Determination of enzymatic (SOD, CAT) and non-enzymatic antioxidants (TRAP).*** Variations in antioxidant levels were observed in all $TiO_2$-particle treated animals at 3 months. As shown in Fig. 9, 150MP, 10NP, and 5NP seemed to induce CAT consumption in lung samples. As regards SOD, mobilization of this antioxidant was only observed in the NP-treated groups. No variations in non-enzymatic antioxidant levels were observed in the experimental groups compared to controls.

As regards liver samples, CAT levels were found to increase significantly in all groups (150MP, 10NP, and 5NP) when compared to controls. Consumption of non-enzymatic antioxidants, as shown by TRAP, was only statistically significant in the $TiO_2$-NP5 group ($p < 0.05$), (Fig. 9).

## DISCUSSION

The toxic effects of different particles have been associated with their physicochemical properties, including size, shape, crystalline structure, chemical composition, area and surface load, porosity, distribution, and aggregation state, among others.[38] The fact that cytotoxicity correlates with particle size would account for the differences in the biological response to the different sized $TiO_2$ particles (150, 10, and 5 nm) observed in the present study.

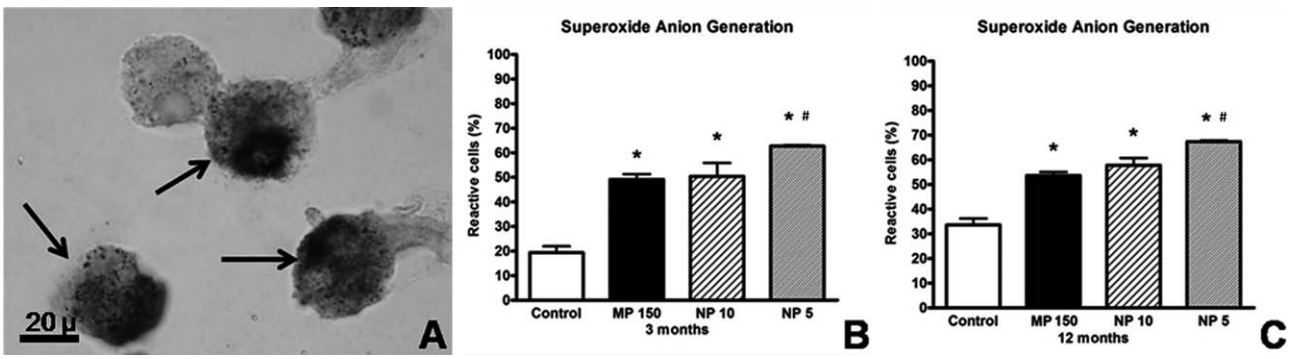


**FIGURE 8**. Superoxide anion ($O_2^-$) generation in lung tissue. (A) Microphotograph of NBT treated alveolar macrophages. Note the difference in the intensity of the stain between reactive (→) and non-reactive cells. Orig. Mag. X1000. (B and C) Percentage of reactive cells post-treatment with $TiO_2$MPs and $TiO_2$NPs, at 3 and 12 months, respectively (mean ± SD, $*p < 0.05$, compared to control; $^{\#}$ $p < 0.05$, for $TiO_2$-NP5 versus $TiO_2$-MP150, $n = 6$).

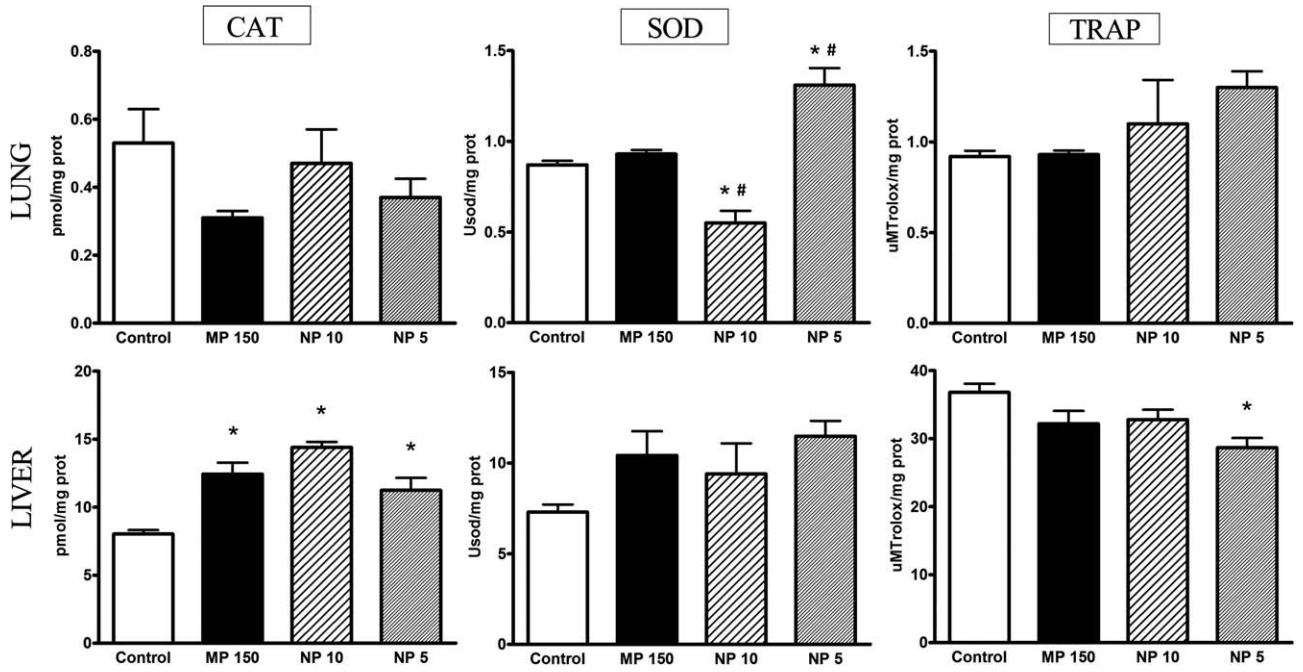


**FIGURE 9**. Determination of CAT, SOD, and TRAP. The graphs show the changes in antioxidant levels in the lung and liver 3 months post-injection. (mean ± SD, $^{*}p < 0.05$, compared to control; $^{\#}p < 0.05$, for NPs versus $TiO_2$-MP150).

The ability of NPs to form larger superstructures of aggregates/agglomerates has been extensively noted in the literature. However, the *in vivo* biological impact of these structures has not been fully assessed.[4,39] The aggregation state and the surface area of some nanoparticulate materials can change once they are introduced into the biological milieu. The levels of particle aggregation should be taken into account when considering size- and dose-dependent toxicity.[40]

The smaller particles showed greater biopersistence in the lung than the larger particles, which would seem to indicate a better capacity of the lungs to clear larger particles. It is known that after entering the body via the respiratory tract, MPs deposit on the surface of the epithelium and are cleared by mucociliary transport and/or macrophage phagocytosis.[41] NPs, however, have the ability to penetrate through the lung epithelium and reach the deeper tissues, but the clearance mechanisms from these deeper tissues remain to be elucidated.[42–46] Ferin et al.[47] found that at equivalent doses, $TiO_2$ -NP20 penetrated the pulmonary interstitium more easily than $TiO_2$ -NP250. The inefficiency of macrophages in the clearance of inhaled NPs prolongs their biopersistence and/or favors their translocation to lung tissues and the blood stream, potentially increasing their adverse health effects.[48] The results obtained in the present study showed that although NPs were more persistent and reactive and led to a greater increase in superoxide anion generation than MPs, they did not cause membrane damage. This phenomenon may be due to the mobilization of antioxidants, which could be an indication of an adaptive response in the lungs. Such a response correlates with the histological finding showing no lung damage. Chen et al.,[49] however, reported that $TiO_2$-NP deposits caused thickening of the alveolar septa and neutrophil infiltrate. In this regard, other studies in the literature have shown the relationship between the surface area of the particle and the severity of its health effects.[50]

The particles found in the liver were inside Kupffer cells and, in agreement with reports in the literature, they were in hepatocytes in some cases.[29,51] Antioxidant activity failed to compensate for membrane damage caused by NP5, which was consistent with the histological findings (necrosis). The results of the present study are in line with studies in the literature reporting similar histological changes.[49,52] Nevertheless, Liang et al.[53] found that NP deposits caused no histopathological changes in the liver. The differences among results could be due to the doses used in each of the studies. Unlike the lungs, MPs showed greater biopersistence in the liver than NP10. In the present study, NP5 were more persistent than NP10, probably due to the capacity of the smaller NP to aggregate and form larger structures that behave like MPs. The significant decrease in NP10 biopersistence may reflect a size-dependent, two-phase cellular response. This response could be elucidated employing particles ranging in size between MP 150 and NP10. The accumulation of particles in the liver and the histopathological and biochemical changes described herein could compromise liver function, as shown by Urban et al.[8]

No significant changes were observed in either liver or lung $TiO_2$-NP5 concentration through time. However, plasma concentration of Ti increased significantly at 12 months, likely due to the translocation of particles to the blood stream from other organs that were not analyzed in this study, such as the spleen and ganglia.

Despite the kidney being exposed to the effect of NPs during ultrafiltration, the impact of NPs remains to be clarified.[4] It is important to note that deposits of NPs were found in the kidney at the longest experimental time point only, but did not cause histological alterations. Nevertheless, some studies found NPs to cause glomerular edema and protein accumulation in renal tubules, as a consequence of retention of $TiO_2$ particles.[49,52] Although histological observation confirmed the presence of particle deposits in kidney sections in all the experimental groups at 12 months, the correlation between the number and size of the deposits and APS could not be established due to the lack of a distinct deposition pattern in each group and of ICP-OES quantitation.

As reported in previous studies conducted at our laboratory,[23] our results showed the presence of mononuclear phagocytic cells loaded with $TiO_2$ particles in the blood, 3 months post-injection. However, titanium was not detected in plasma until 12 months post-injection. Thus, blood cells loaded with Ti might serve as early bioindicators of corrosion.

As regards oxidative metabolism, all $TiO_2$ particles caused an increase in superoxide anion generation in AM compared to controls, at all the experimental time points. Although superoxide anion generation tended to increase with particle size, the differences between groups only reached statistical significance when comparing $TiO_2$-NP5 and $TiO_2$-MP150. Our results are in line with a number of studies showing that the smaller the particle, the greater its toxicity.[1,38,54–56] Although $TiO_2$-NP5 concentration in the lungs was significantly lower as compared to the other studied particles, they caused a significantly higher increase in superoxide anion generation but no histological alterations.

Superoxide anion levels were higher in older (12 months) than in younger rats (3 months). It is well documented that basal oxidative metabolic rate is elevated and oxidative metabolic response to a toxic agent is lower in older adults.[57,58] The biological effect of the particles would therefore be more adverse in this age-group, which is one of the sectors of the population that is in most need of metallic medical implants. It must be pointed out that most implants are placed in older adults who often have a mild insufficiency (heart, kidney, or respiratory). This must be taken into account since the effect of NPs could have clinical implications, particularly because they can disseminate through the body. With the aims to complement the present study and elucidate possible functional alterations, further research will be conducted at our laboratory to analyze the functional capacity of the organs studied here.

As regards antioxidant enzymes, response to the increase in superoxide anion generation caused by titanium particles seems to be mediated by SOD activity in lung tissue and by CAT activity in the liver. Unlike CAT response in the liver, SOD activity in lung tissue was APS-dependent. CAT activity in the liver, however, increased in response to the presence of particles in the tissue, but not to particle size.

Understanding the biokinetics and the potential health risks of particles that could be released from the surface of a titanium medical implant is a challenge to nanotoxicology and biocompatibility studies. The present study contributes clinically relevant scientific information on the biokinetics and possible adverse biological effects of different sized $TiO_2$ particles on host tissues.

## ACKNOWLEDGMENTS

This work was supported by grants: UBACyT 20020100200157 and 20020100100812 from the University of Buenos Aires, PICT 2008-1116 from the National Agency for the Promotion of Science and Technology, and PIP 11220090100117 (National Council for Scientific and Technological Research). Argentina. We would like to extend special thanks to Ms. Mariela Domingo, student research fellow of the School of Dentistry of the University of Buenos Aires, for her collaboration in performing the study.

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