

# An experimental study of the dissemination of Titanium and Zirconium in the body

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Metallic implants can generate and release titanium oxide (TiO<sub>2</sub>) and zirconium oxide (ZrO<sub>2</sub>) to the tissues. These products can accumulate locally or disseminate systemically.

The aim of the present study was to assess the distribution of TiO<sub>2</sub> and ZrO<sub>2</sub> administered intraperitoneally to rats.

We used male Wistar rats of approximately 100 g body weight throughout the study. An intraperitoneal injection of a suspension of TiO<sub>2</sub> or ZrO<sub>2</sub> (16, 1600 and  $16 \times 10^3$  mg/kg body weight) was administered. The animals were killed at 5–10 months post-administration by ether overdose. Samples of peritoneum, liver, kidney, lung and spleen were taken, fixed in formalin and routine processed for embedding in paraffin. One set of sections was stained with hematoxylin and eosin and another set was prepared unstained. The presence of titanium in the tissues was detected by X-ray diffraction crystallography.

The histological analysis revealed the presence of abundant intracellular aggregates of metallic particles of Ti and Zr in peritoneum, liver, lung and spleen. The crystallographic study revealed the presence of anatase. The dissemination of metallic particles from orthopedic or odontological implants would not be restricted to a local phenomenon. The particles also target vital organs. The distribution of these deposits over lengthy periods deserves meticulous attention given the clinical relevance of this phenomenon.

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## Introduction

The use of different metals in the construction of odontological and orthopedic implants has become widespread [1].

The implant is placed in bone tissue and gives rise to the process of osseointegration [2].

There may be a material between the metal and the tissue. Such is the case of hip prostheses cemented with methyl methacrylate, where osseointegration does not occur. Areas of the metal implant may interact with one another or with plastic material such as high impact polyethylene [3] resulting in the formation of metallic or plastic debris. The debris falls into the articular cavities and ultimately reaches the tissues and induces a granulomatous reaction [4].

Of all the materials from which implants are made,

titanium and zirconium are the most frequently used in direct contact with tissue, be it bone or otherwise. These metals (atomic weight of Titanium (Ti): 47, atomic weight of Zirconium (Zr): 91) have a feature in common. On exposure to air or liquid such as water they develop a layer of oxide that makes them unreactive [5, 6]. This is the layer that effectively comes into contact with the tissue when these materials are used as implants.

It is thus of interest to study the behavior of titanium oxide (TiO<sub>2</sub>) and zirconium oxide (ZrO<sub>2</sub>) in contact with tissues. It is particularly relevant to study their dissemination in the body and their deposition in critical organs such as liver, lung, kidney and spleen. The aim of this study was to develop a model to assess the distribution of these oxides (TiO<sub>2</sub> and ZrO<sub>2</sub>) administered intraperitoneally to the rat.

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## Materials and methods

Male Wistar rats of approximately 100 g body weight were used throughout. We followed the NIH Publication #85-23 (rev. 1985) for the care and use of laboratory animals. A single intraperitoneal injection of a suspension of  $\text{TiO}_2$  (anatasa, Sigma Chemical Company) ( $n=14$ ) or  $\text{ZrO}_2$  (Fluka Chemie AG-Switzerland) ( $n=9$ ) was administered at doses of 16, 1600 or  $16 \times 10^3$  mg/kg body weight.

The sizes of the  $\text{TiO}_2$  and  $\text{ZrO}_2$  particles were about  $1 \mu$ , and they showed a sphere-like shape. A control group of animals ( $n=10$ ) was given an intraperitoneal injection of equivalent volumes of saline solution to evaluate the effect of the vehicle. Both groups were allowed to live for 5–10 months. The animals were then killed by ether overdose. Systematic autopsies of all the animals were performed. Samples of peritoneum, liver, kidney, lung and spleen were fixed in 10% neutral buffered formalin, embedded in paraffin and sectioned. One set of sections was stained with hematoxylin and eosin and another set was prepared unstained. In all the cases, the sections were treated with a saturated solution of picric acid to remove formalin pigments. In the cases in which the macrophages were loaded with metallic particles the sections were stained with PAS.

Samples of liver, lung, peritoneum, kidney and spleen were submitted to enzymatic digestion with trypsin to evaluate the presence of titanium and perform the corresponding crystallographic characterization. The sediment obtained by enzymatic digestion was submitted to crystallographic analysis by X-ray diffraction.

## Results

None of the experimental or the control animals showed alterations in body weight, behavior or general health.

The analysis of the necropsies of the animals injected with low doses (16 and 1600 mg/kg body weight) of  $\text{TiO}_2$  or  $\text{ZrO}_2$  failed to reveal alterations. The peritoneum of the animals injected with the higher dose ( $16 \times 10^3$  mg/kg body weight) exhibited whitish deposits in the case of  $\text{TiO}_2$  and gray deposits in the case of  $\text{ZrO}_2$ . We failed to detect areas of adherence between peritoneum and intestine. None of the cases showed granulation tissue. Liver, spleen and lung failed to reveal macroscopic alterations.

## Histological studies

Formalin pigments were not detected.

**Peritoneum.** Aggregates of macrophages loaded with particles that occupied most of the cytoplasm were detected, regardless of the dose and oxide injected. Despite the fact that the deposits were abundant, there was no formation of giant cells or significant neovascularization activity.

**Liver.** In all the cases under study, macrophages loaded with particles were a frequent finding, regardless of the dose of oxide administered (Fig. 1(A), (B)). Most of the macrophages were found in the vicinity of the sinusoid capillaries and in the Kiernan spaces. The liver surface failed to exhibit macrophages. In some of the animals injected with the highest dose ( $16 \times 10^3$  mg/kg

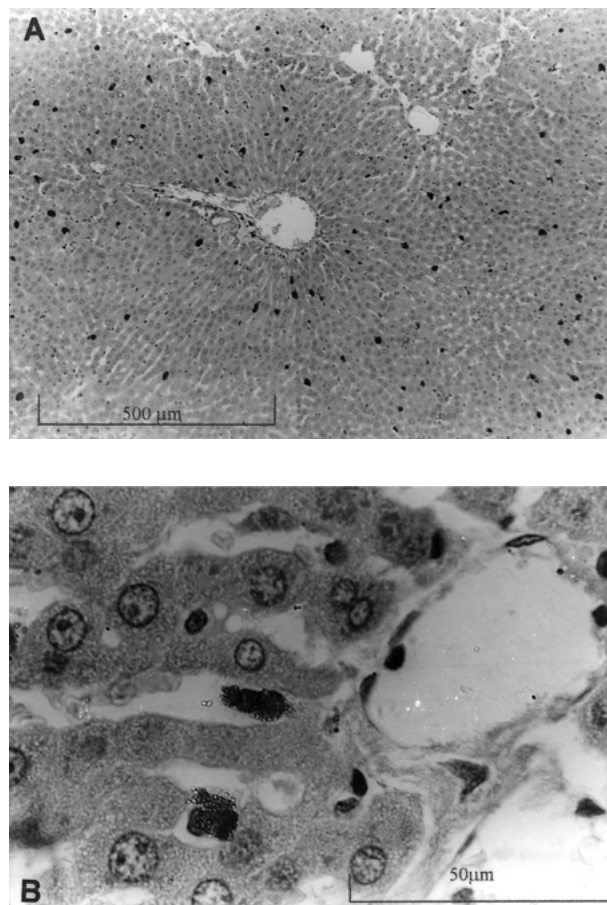


Figure 1 (A) Liver: Aggregates of material diffusely distributed can be observed ( $\text{TiO}_2$ ) (H-E). (B) At a higher magnification macrophages loaded with particles can be clearly identified in the vicinity of sinusoid capillaries.

body weight) of  $\text{TiO}_2$  or  $\text{ZrO}_2$  a greater macrophagic activity was detected at 5 months in the Disse space.

**Lung.** Foci of alveolar macrophages loaded with particles were found (Fig. 2). In one of the cases injected with  $\text{TiO}_2$  (16 g/kg body weight) we observed an inflammatory reaction in an area of lung parenchyma adjacent to the area occupied by macrophages.

**Spleen.** Foci of macrophages were detected, particularly in the splenic cords of Billroth (Fig. 3).

**Kidney.** The tissue appeared normal.

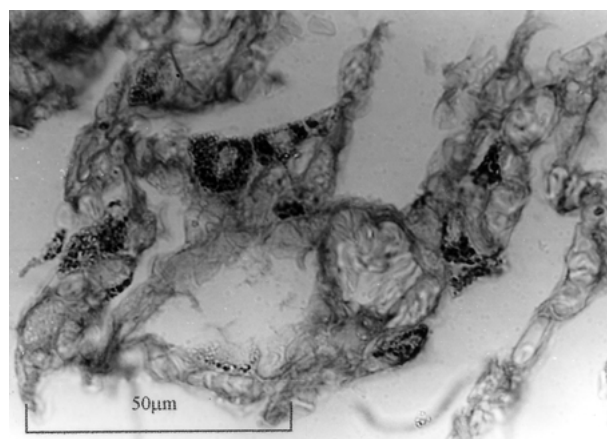


Figure 2 Lung: Alveolar macrophages densely loaded with particles can be observed (H-E).

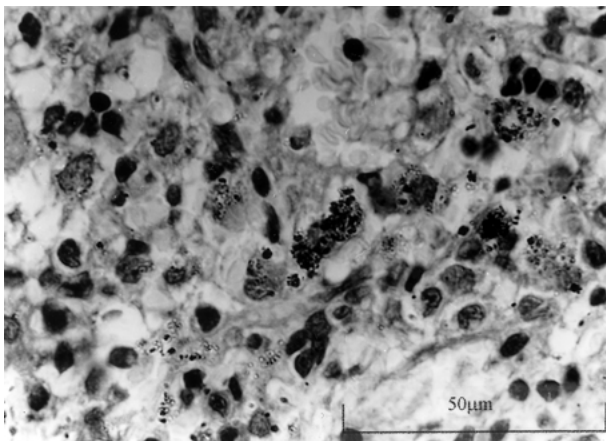


Figure 3 Spleen: Abundant macrophages can be observed in the splenic cords of Billroth (H-E).

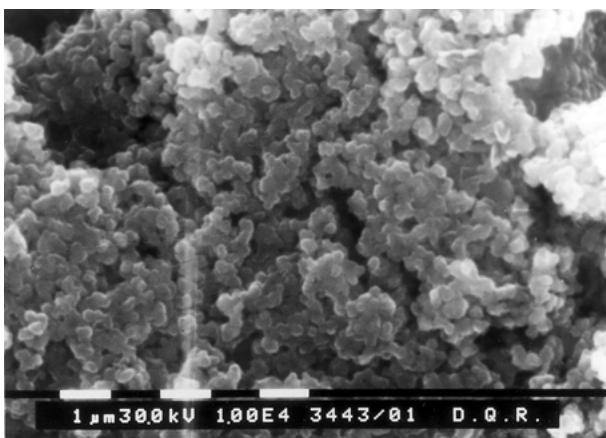


Figure 4 SEM: Note the presence of particles of  $\text{TiO}_2$  that result from the enzymatic digestion of the tissues under study.

The macrophages loaded with particles exhibited an intense PAS reaction.

### X-ray diffraction

The study of the sediment obtained after enzymatic digestion of the tissues revealed the presence of  $\text{TiO}_2$  (anatasa) (Figs. 4 and 5).

### Discussion

The use of Ti and Zr is accepted in orthopedic and odontological rehabilitation. In this context, the data presented herein pose queries regarding the possibility of the dissemination of the Ti and Zr oxides to critical organs.

Urban *et al.* [7] showed dissemination of metallic and plastic particles from hip and knee replacements to organs such as liver, spleen and lymph nodes in 27 autopsies. Furthermore, the same authors state that “there have been several epidemiological studies of cancer incidence in the first and second decades following total hip replacement” [7].

Other studies report the presence of macrophages or granulation tissue in the vicinity of failed prostheses, be they orthopedic or odontological [8–13].

We have previously shown that titanium particles are

released by a large proportion of the odontological implants that have failed due to mobility. These titanium particles are phagocytosed by macrophages in the periprosthetic soft tissues [14].

We herein present an experimental model that involves dissemination and deposition of  $\text{TiO}_2$  and  $\text{ZrO}_2$  particles in liver, lung, spleen and peritoneum. We particularly draw attention to the presence of particles in the lung given that this issue could be explored clinically.

The literature on other metals such as nickel, chromium and cobalt describe transport mechanisms that involve binding of these metals to blood cells and/or proteins, particularly albumin [15,16]. Aluminum has been reported to disseminate in plasma bound to transferrin [17].

Different possible mechanisms of dissemination have been described in the literature for titanium, i.e. systemic hematogenous dissemination, either in solution or as particles [18]; via the lymphatic system as free particles or phagocytosed within macrophages [7, 19]; dissemination to bone marrow via circulating monocytes or as small particles in the blood stream [20]. There are hypotheses regarding the mechanisms of dissemination of titanium. However, little is known about the valency with which titanium interacts, the organic or inorganic nature of its ligands and its potential toxicity [21]. The present study demonstrates that the injected  $\text{TiO}_2$  is deposited as such in the organs examined and preserves identical crystallographic characteristics (anatasa). The liver surface fails to show macrophages, thus suggesting that the deposition of  $\text{TiO}_2$  or  $\text{ZrO}_2$  in the parenchyma of this organ would occur via vascular dissemination. The presence of these oxides in alveolar macrophages would occur by the same mechanism.

The usual way in which metals are deposited is classically associated to the production of mucoproteins as revealed by the PAS-positive reaction of the macrophages analyzed herein.

Titanium and Zirconium undoubtedly disseminate actively in the body. However, these deposits do not seem to trigger short-term granulomatous reactions.

These data show that the dissemination of metallic particles from orthopedic or odontological implants is not a purely local concern. Moreover, dissemination is not restricted to vascular transport and elimination through the kidney, i.e. these products are deposited in target organs. In this context, future studies on the long-term follow-up of these deposits and their ultimate fate are warranted.

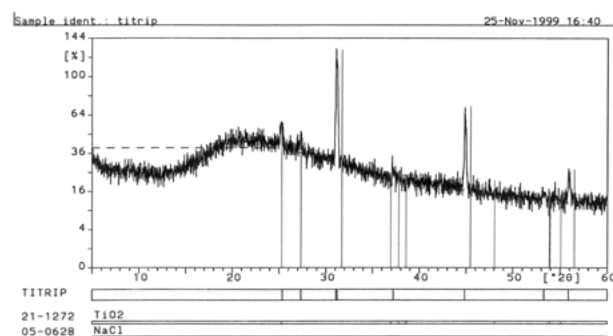


Figure 5 X-ray diffraction pattern showing the presence of  $\text{TiO}_2$  (anatasa) in the particles analyzed.

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## References

1. B. D. RATNER, in "Biomaterials Science: An Introduction to Materials in Medicine" (Academic Press, Inc., 1996) p. 1.
2. T. ALBREKTSSON, *J. Prosthet. Dent.* **50** (1983) 255.
3. A. KOBAYASHI, W. BONFIELD, Y. KADOYA, T. YAMAC, M. A. FREEMAN, G. SCOTT and P. A. REVELL, *Proc. Inst. Mech. Eng. (H)* **211** (1997) 11.
4. M. T. MANLEY, in "The Art of Total Hip Arthroplasty" (Grune & Stratton, Inc., 1987) p. 257.
5. B. KASEMO, *J. Prosthet. Dent.* **49** (1983) 832.
6. D. F. WILLIAMS and R. L. WILLIAMS, in "Biomaterials Science: An Introduction to Materials in Medicine" (Academic Press, Inc., 1996) p. 260.
7. R. URBAN, J. JACOBS, M. TOMLINSON, J. GAVRILOVIC, J. BLACK and M. PEOCH, *J. Bone Joint Surg.* **82-A** (2000) 457.
8. V. G. LANGKAMER, C. P. CASE, P. HEAP, A. TAYLOR, C. COLLINS, M. PEARSE and M. SALOMON, *ibid.* **74B** (1992) 831.
9. J. M. LEE, E. A. SALVATI, F. BETTS, E. F. DICARLO, S. B. DOTY and P. G. BULLOUGH, *ibid.* **74-B** (1992) 380.
10. P. A. REVELL, in "Bone and Joint Disease" (Springer-Verlag, 1982) p. 73.
11. L.-E. MOSBERG, A. NORDENRAM and O. KJELLAMAN, *J. Oral Maxillofac. Surg.* **18** (1989) 311.
12. J. C. KELLER, F. A. YOUNG and B. HANSEL, *Dent. Mater. J.* **1** (1985) 41.
13. K. BESSHO, K. FUJIMURA and T. IIZUKA, *J. Biomed. Mat. Res.* **29** (1995) 901.
14. D. OLMEDO, M. M. FERNANDEZ, M. B. GUGLIEMOTTI and R. L. CABRINI, *J. Dent. Res.* **78** (1999) 932.
15. K. MERRITT, S. A. BROWN and N. A. SHARKEY, *J. Biomed. Mater. Res.* **18** (1984) 1005.
16. S. A. BROWN, K. MERRIT, L. J. FARNSWORTH and T. D. CROWN, in "Quantitative Characterization and Performance of Porous Implants for Hard Tissue Applications" (American Society for testing Materials, 1987) p. 163.
17. A. C. ALFREY, in "Aluminum Health. A Critical Review" (H. J. Gitelman, 1989) p. 101.
18. G. MEACHIM and D. F. WILLIAMS, *J. Biomed. Mater. Res.* **7** (1973) 555.
19. P. D. BIANCO, P. DUCHEYNE and J. M. CUCKLER, *Biomaterials* **17** (1996) p. 1937.
20. C. A. ENGH, JR, K. D. MOORE, T. N. VINH and G. A. ENGH, *J. Bone Joint Surg.* **79-A** (1997) 1721.
21. J. JACOBS, M. D. SKIPOR, J. BLACK, R. M. URBAN and J. O. GALANTE, *ibid.* **73-A** (1991) 1475.

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