Titanium levels in rats implanted with Ti6Al4V treated samples in the absence of wear

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The effect of implantation time and implant nitriding on titanium ion concentration in several tissues of rats carrying Ti6Al4V implants was studied by means of inductively coupled plasma-mass spectroscopy (ICP-MS). Histological studies were also performed in order to check for tissue degeneration due to the Ti6Al4V implantation. The animals were divided into four groups: one received Ti6Al4V implants, the second received nitrided Ti6Al4V implants, the third group received nitrided and descaled Ti6Al4V implants and the last one was the control group. Half the animals of the implanted groups received the Ti6Al4V implant for 30 days, while the other half received the implant for 120 days. Spleen, muscle, kidney, lung, brain and bone samples were retrieved from these rats as well as the control group. Ion concentration measures did not show significant differences between control and implanted rats for the studied period of time, although histological studies showed minor differences, especially on liver tissue samples.

1. Introduction

Titanium and Ti6Al4V alloy have been recognized as metals with an excellent biocompatibility. However, literature information concerning the titanium ion levels in tissues associated with the use of Ti6Al4V 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 and retrieval used in each study.

Woodman *et al.* [1] used baboons as an animal model and found that there were no statistical differences in the titanium level in tissues between implanted and control animals. Lugowski *et al.* [2] studied the effect of implanting porous and solid Ti6Al4V samples in rabbits. The results did not show statistical differences in titanium ion levels between the two sample groups. Bianco *et al.* [3] also used rabbits as an animal model. Porous Ti6Al4V samples were implanted into the tibia of the rabbits. Measurements of the evolution in time of titanium ion levels in tissue samples did not show statistically significant increments, nor in comparison with control groups.

The present study is focused on the effects of the ion concentration in tissues, combining chemical analysis techniques as inductively coupled plasma-mass spectroscopy (ICP-MS) with histological studies of tissue samples. The effect of implantation time and the use of nitrided Ti6Al4V extra-low interstitial (ELI) implants (a thermochemical treatment for improvement of surface hardness) on titanium ion concentration in tissues were also studied.

2. Materials and methods

Ti6Al4V ELI was chosen as implant material. On reception the material presented a surface hardness of $315\,H_v$, a mill-annealed microstructure and a chemical composition in compliance with ASTM F136 standard. The implant geometry corresponded to cylindrical plates, 10 mm diameter and 1 mm thickness. The samples were descaled and polished with $0.05\,\mu$m alumina powder suspension, in order to achieve a measured surface roughness of $R_a < 0.50\,\mu$m.

The implants were divided in three groups of six implants each: non-treated samples, nitrided samples and descaled nitrided samples. Thermochemical nitriding of Ti6Al4V was obtained by heating the samples at 900 °C during 60 min in a high purity nitrogen atmosphere (nitrogen impurities were below 5 p.p.m) [4]. Descaling affected about $4\,\mu$m of the material surface. After

treatment, selected samples were studied by X-ray diffraction, using CuK_α radiation and electron diffraction patterns in order to characterize surface nitrided films. Prior to implantation, samples were prepared following ASTM standard guidelines for surgical implants [5].

Twenty-one adult male Wistar rats, weighing 125–150 g, were divided into four groups, three groups of six animals that received an implant and one control group of three animals. National Institutes of Health (NIH) guidelines for care and use of laboratory animals were used through the experiment [6]. The animals were anaesthetized with isofluorane at 5% vaporized in O_2 flowing at $2\,l\,min^{-1}$ for implantation and sample retrieval. Animals were connected to the circuit with a modified Hall mask. Once anaesthetized, an incision was made in the lower third of the left paravertebral region in aseptic conditions. Muscle fibers were separated in order to implant the sample inside the muscle. Sample migration was avoided closing the fascia with a non-resorbable suture point.

In each group of animals, three animals received an implant for 30 days and another three rats received an implant for 120 days. Control data were obtained from the group of three rats that did not receive an implant. The average implant surface area to rat weight was about 8 times the ratio a standard person with a cemented hip arthroplasty would experience. The animals neither died during the experiment nor showed signs of illness.

After an appropriate implantation time, the rats were euthanized. During necropsy, spleen, contralateral and paravertebral muscle, kidney, lung, brain and bone samples were retrieved from the animals and weighed, as well as the implants. After extraction, organs were divided in two. One half was weighed, frozen at $-80\,^{\circ}C$ in liquid nitrogen and stored until analysis with ICP-MS. The other half of each organ was used for histological studies, as described below.

ICP-MS analyses of tissue samples requires the sample to be in liquid form, so the samples were prepared after thawing by means of microwave digestion, obtaining a homogeneous liquid. Histological samples for light microscopy were routinely fixed in 10% neutral buffered formalin and dehydrated in graded concentrations of ethanol and embedded in paraffin according standard methods. Serial sections were cut at 7 μm thickness and stained with Haemalum and picroindigo-carmine. The stained samples were then analyzed.

3. Results

Surface hardness of nitrided samples increased up to 300% (over $1000\,HV_{0.1}$) compared to non-treated samples. X-ray diffractograms of the treated samples (Fig. 1) showed the existence of a surface titanium nitride layer of 2–4 μm width, consisting mainly of ε-nitride (Ti_2N) with some δ-nitride (TiN). A layer of highly nitrogen-enriched α-titanium layer of several micrometers width was detected under the nitrided layer.

Measurements made with sample area diffraction pattern (SADP) confirmed the existence of Ti_2N with measured lattice parameters of $a = 0.468\,nm$ and $c = 0.295\,nm$, close to the standard values of ε-nitride

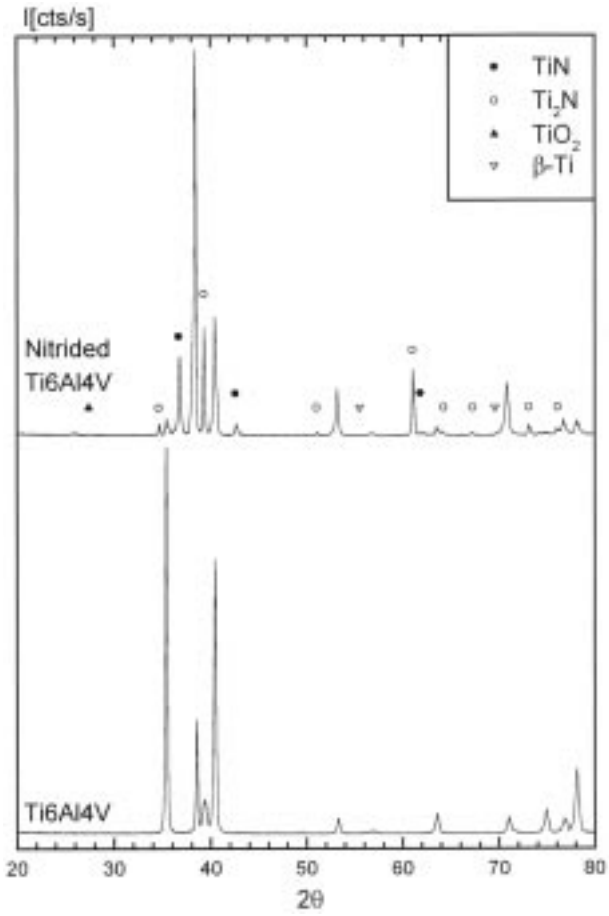

Figure 1 Ti6A14V and nitrided Ti6A14V diffractograms.

($a = 0.4943\,nm; c = 0.3036\,nm$) [7]. Descaled sample observations showed the existence of subnitrides and distortion of the α-titanium lattice parameters, probably due to the presence of nitrogen into the lattice. When the implants were retrieved it was observed that they were encapsulated in connective tissue, surrounding both the treated and non-treated implants.

ICP-MS of the titanium ion concentration on tissues did not show significant differences between control and implanted rats or between rats implanted for different periods of time (Table I), in agreement with the above mentioned references [1–3]. No statistically significant differences on titanium ion concentration were observed between rats implanted with different kinds of materials. Slight increments on samples taken at 30 days were observed in liver, although titanium ion levels returned to values similar to control ones on samples taken at 120 days. The highest of titanium ion levels were measured in spleen samples.

Histological studies with optical microscopy of muscle, lungs, brain and bone tissue samples did not show any differences between control and implanted rats. However, slight differences of some tissues when compared control and the three groups of implanted rats were observed. Specifically, spleen tissues of the implanted rats showed an increase in plasmatic antibody secreting plasma cells (Fig. 2) forming groups of cells when compared to control samples (Fig. 3). These differences could be due to an unspecified reaction and not related to material implantation.

TABLE I Titanium ion levels in sample tissues ($n = 3$)

Sample	Content (ng ml^{-1})														
	Lung			Muscle[a]			Kidney			Spleen			Liver		
	0	30	120	0	30	120	0	30	120	0	30	120	0	30	120
Control	740 ± 54			1060 ± 142			882 ± 34			1659 ± 170			850 ± 30		
Ti6A14V Untreated		857 ± 21	895 ± 19		–[b]	1130 ± 107		892 ± 30	913 ± 29		1356 ± 26	1370 ± 28		1021 ± 41	891 ± 14
Treated Ti6A14V		812 ± 25	801 ± 21		–[b]	1079 ± 70		887 ± 20	863 ± 44		1370 ± 40	1381 ± 38		1018 ± 41	805 ± 32
Treated and descaled Ti6A14V		879 ± 23	888 ± 23		921 ± 56	1035 ± 35		963 ± 17	899 ± 51		1353 ± 18	1316 ± 63		1036 ± 30	937 ± 39

[a] Contralateral muscle.
[b] Invalid results due to preparation problems.

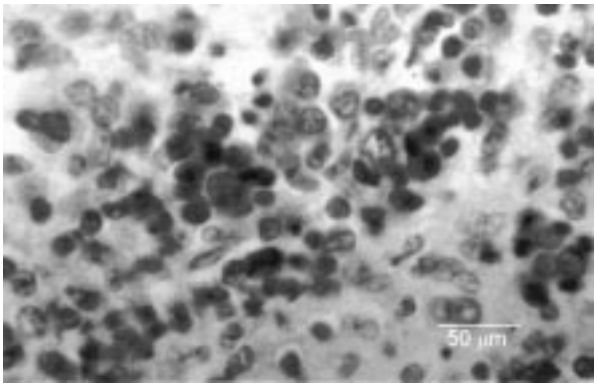

Figure 2 Groups of cells in spleen tissue of a Ti6A14V implanted rat.

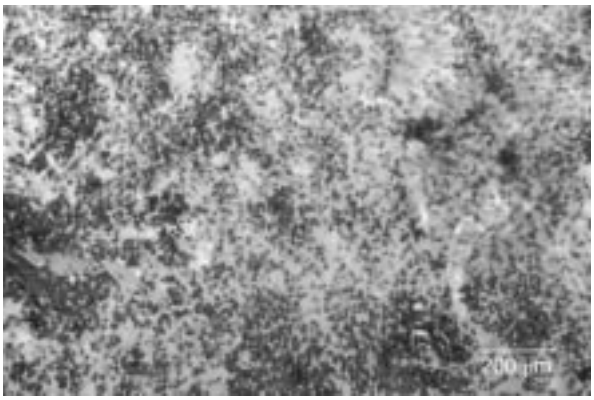

Figure 3 Spleen tissue of a control rat.

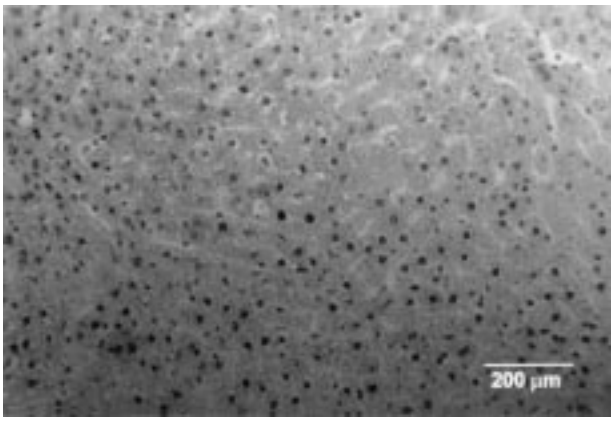

Figure 4 Liver sample of an untreated Ti6A14V implanted rat with cell vacuolization.

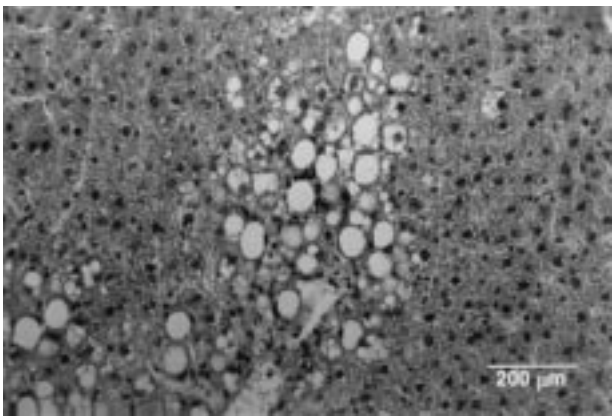

Figure 5 Liver sample of an untreated Ti6A14V implanted rat with cell degeneration.

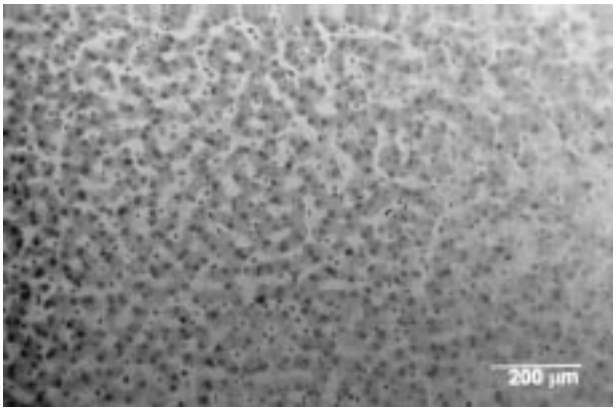

Figure 6 Liver tissue of a control rat.

However, the main differences were observed on liver samples, with signs of vacuolization of some cells (Fig. 4) and symptoms of cell degeneration of the hepatic parenchyma (Fig. 5) when compared to control samples (Fig. 6).

4. Discussion and conclusions

The tissues samples examined with ICP-MS did not show evidence of systemic accumulation of titanium ions due to implantation of a Ti6A14V sample. The values obtained are consistent with those of literature, considering the different animal models and exposed surface/weight ratio. The main differences observed were found when control groups were compared to implanted rats, although these slight variations are probably due to the implantation process. These results are valid for both treated and untreated Ti6A14V implants.

The stability of the measured ion levels cannot be linked to the position of the studied tissue samples relative to the implant, as the studied contralateral muscle tissue, very close to the implant, neither presented an increment of titanium ion levels with time when compared to the measurements of samples taken from implanted and unimplanted rats.

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. The evidences of alterations of spleen observed with histological observations may not be related to the implantation of Ti6A14V samples, but to an immune response of the organ. The observations suggest the existence of an immune response in spleen, although probably this effect is not due to a toxicity effect of the implant. However, the evidences of liver cell degeneration, although not conclusive, are not as easily explained as the spleen ones, and could be due to some kind of toxic effect.

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References

1. J. L. WOODMAN, J. J. JACOBS, J. O. GALANTE and R. M. URBAN, *J. Orthop. Res.* **1** (1984) 421.
2. S. J. LUGOWSKI, D. C. SMITH, A. D. MCHUGH and J. C. VAN LOON, *J. Biomed. Res.* **25** (1991) 1443.
3. P. D. BIANCO, P. DUCHEYNE and J. M. CUCKLER, *J. Mater. Sci.: Mater. Med.* **8** (1997) 525.
4. D. RODRÍGUEZ, F. J. GIL and J. A. PLANELL, *J. Biomech.* **31** (1998) 49.
5. ASTM F86, Standard recommended practice for surface preparation and marking of metallic surgical implants, ASTM (1994).
6. NIH publication 85–23, National Institutes of Health, USA (1985).
7. H. A. WRIEDT and J. L. MURRAY, *Bull. Alloy Phase Diagr.* **8** (1987) 378.

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