PIXE micro-beam mapping of metals in human peri-implant tissues

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Previous investigations did not agree about the possible presence of titanium and other metals in the tissues around endosteal dental implants and joint prostheses. Indeed, while some authors reported diffusion of metals into the tissues, some others did not find evidence of this phenomenon. In the present study, four dental titanium implants, removed with the surrounding tissues from patients at various time intervals after the insertion, were studied by means of the micro-beam proton-induced X-ray emission (PIXE μ-beam) technique, which draws maps showing the tissue distribution of elements with a detection limit of about 1 ppm.

One implant was built in commercially pure titanium, two others in titanium coated with titanium plasma spray, and the fourth in Ti–Al–V alloy. Their composition was confirmed by the PIXE μ-beam analyses.

The removed samples were embedded in epoxy and processed with a cutting–grinding appliance, mounted on plastic holders, and ground up to a thickness of about 35 μm. Optical microscope examinations were also carried out, to compare the optical findings with the elemental maps obtained with the PIXE μ-beam.

One implant, removed after 70 days because the patient had developed peri-implantitis, had some inflammatory soft tissue attached, with no evidence of metal leakage. The other three implants had been removed after 6, 7 and 9 years of valid clinical service, because of the fracture of the prosthetic abutment or the implant stem. At the optical microscope, all these fixtures were embedded in mature bone.

The elemental maps indicated small titanium deposits in about 5% of the bone bordering the implants, while aluminum, when present in the fixture, leaked diffusely into the surrounding bone and vanadium was not found in the tissues.

These results suggest that titanium may be found occasionally in peri-implantar tissues, but has very little tendency to spread, while the presence of aluminum in the implant alloy may cause an important leakage of this metal.

Introduction

Commercially pure (CP) titanium and Ti–6Al–4V and similar alloys are commonly used in building dental and orthopedic implants. Titanium has proved to be one of the best materials for this purpose, due to its excellent biocompatibility, that in most cases leads to the so-called osteointegration, and guarantees easy construction of surgical prostheses.

Even if titanium is considered biologically safe by many authors, some possible adverse effects have been pointed out. When widely inhaled as powder, titanium was found to induce mutations in the alveolar cells of rats [1]; however, studies carried out on workers exposed to TiO_2 inhalation showed no significant clinical damage [2, 3].

An *in vitro* inhibition on the growth of bone cells and fibroblasts was described [4]. Also an *in vitro* alteration of calcium deposition, caused by titanium and vanadium ions, was reported [5]. Though cytotoxicity of pure titanium on human cells cultures was found [6], Ti

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showed the lowest toxicity among the metals and alloys tested in the same experiment.

Wang *et al*. (1997) [4] described impairment of some immune factors in rats which had been injected with titanium particles into the peritoneum. After a peritoneal injection in rats, titanium concentrated in tissues and organs, especially spleen and kidney, for up to 20 days [7].

A generic foreign body reaction to implanted metals, including titanium, was also reported as possible when they are inserted in the abdomen of rats [8]. A few authors suspect [9] that titanium may cause major damage to the organism, even to the extent of being carcinogenic, but supporting evidence has yet to be found.

Despite its natural passivation due to the thin oxide layer that naturally forms on its surface, when immersed in simulated body fluid or saline solution titanium releases ions [10, 11]; this phenomenon can be minimized, though not completely avoided, by passivating treatments, such as nitric acid treatment, anodization [12] or aging by boiling in de-ionized water; mechanical wear and surface roughness seem to enhance the metal's release.

Other researchers found that titanium alloys have a greater *in vitro* ion release than that of Co–Cr alloys, in spite of the generally accepted lower biocompatibilty of the latter [13]. The possibility that titanium and other metals release ions into the tissues was first pointed out by Ferguson *et al*. (1962) [14], who found increased levels of metal ions both in the peri-implant tissues and in the organs of rabbits, that had undergone surgical insertion of titanium and other alloys.

Several authors found noticeable amounts of Ti in peri-implant human tissues [15–17] and Ti levels were increased in patients with prosthetic hip replacements [18]; similar high levels of titanium following bone implantations were found around titanium implants in dogs [19]; and even in various organs, such as the lungs, of animals which had undergone surgical insertion of Ti implants, especially as orthopedic devices [20]. Ti accumulation was found by Schliephake *et al*. [22] around plates for the treatment of fractures in humans; the same author, after inserting Ti screws in the mandible of animals, found metal particles in the tissues surrounding the implant, and a concentration of Ti in lungs. A similar finding was reported by Weingart *et al*. [23], who detected Ti particles in the regional lymph nodes of dogs with Ti plasma-spray (TPS) coated implant screws.

A three-fold increase of Ti concentration in serum and urine was reported in patients with hip prostheses containing Ti, while patients bearing Cr–Co alloy showed a five- to eight-fold increase of serum and urine chromium concentration [24].

On the other hand, Lugowski [25] found very little or no increase of Ti level in the organs of rabbits treated with implants and, similarly, Rodriguez [26] could not find abnormal levels of titanium either in the peri-implant tissues or in the organs of rats.

Bianco *et al*. [27] reported that titanium does not increase in the serum and urine of rabbits after bone implantation; in a similar investigation, no increase in the level of Ti was found in rabbit's organs, as compared to control animals [28]. The same authors, however, found increased Ti levels in the peri-implant bone and muscles [29]. These authors conclude that titanium may be released by implants in some instances, but it tends to stay locally, due to its very low solubility.

This study aims at investigating the possible release of Ti and its distribution in the peri-implant tissues of humans, and was carried out by collecting some implants from patients, who had the implant removed at different time intervals since insertion, from 2 months to 7 years. Removal was required because of peri-implantitis or fracture of the prosthetic abutment.

Materials and methods

Micro-beam proton induced X-ray emission (PIXE μ-beam) was used to search for Ti and other elements in the tissues surrounding the implants; this micro probe technique preserves the topographic relationship between the implant and the tissues, and performs the mapping of the trace elements, thus detecting where metal may have been released.

This investigation was carried out on four titanium implants, removed from four patients at different times. One implant was a CP smooth titanium device, two were titanium coated with TPS devices and the fourth was a Ti–Al–V alloy device, as shown in Table I.

Implant no. 1 (Fig. 1(a)) was a smooth titanium CP fixture which served as abutment for a single upper bicuspid replacement, single-screwed to the implant, which broke after 6 years of clinical service in a 55-year-old man. The implant was removed in order to insert a new fixture, after a bone graft.

Implant no. 2 (Fig. 1(b)) had been placed 9 years before to replace an upper incisor. It was a cylindrical TPS coated screw, the removal of which was needed because of the fracture of the screw and the impossibility to remove and substitute the post, that had been apparently screwed with cement, as in the first specimen. The patient was a 44-year-old female.

Implant no. 3 (Fig. 1(c)) was a bulk screw implant, *en bloc* with the abutment, which had been inserted in the position of a mandibular cuspid, 7 years before, supporting a removable overdenture with another similar implant on the other side, also in canine position. The

TABLE I Investigation on four titanium implants, removed from four patients at different times

Implant	Location	Time of removal	Cause of removal	Implant type
1	Upper maxilla, lateral	6 years	Fracture of abutment	CP Ti screw
2	Upper maxilla, frontal	9 years	Fracture of abutment	Ti, TPS coated, cylinder
3	Chin	7 years	Fracture of abutment	Ti–Al–V, screw
4	Lower maxilla, lateral	70 days	Peri-implantitis	Ti, TPS coated, hollow cylinder

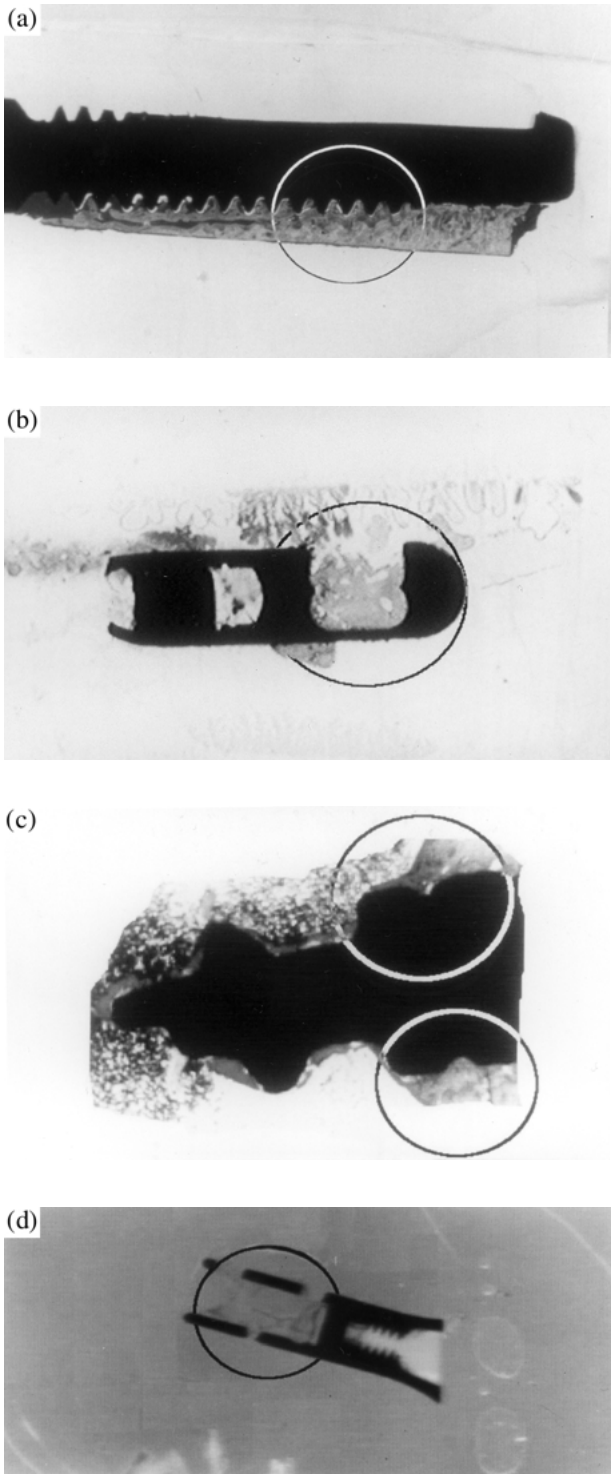


Figure 1 (a)–(d) The retrieved implants: circles point out the areas where proton microbeam scanning was carried out.

fracture of the transmucosal post caused a gingival pocket with recurring inflammation, that suggested removal of the implant in a 61-year-old male.

It must be noticed that implants 1, 2 and 3 n. 2 had been placed by different operators from those who removed them.

Implant no. 4 (Fig. 1(d)) was a cylindrical hollow titanium TPS coated fixture, inserted in the position of a first molar in the lower maxilla, that had to be removed after 70 days from insertion in a 63-year-old female patient, because of peri-implantitis and mobility.

The removal was carried out carefully, using low speed burs under saline irrigation and hand chisels, taking care to save the tissue attached to the fixture. Preserving the surrounding tissues was much easier in the three implants removed because of the fracture of metal components, as in these cases a fair amount of apparently calcified tissue remained attached to the metal. In the other case, where the fixture was loose and embedded in inflammatory soft tissue, it was possible to preserve only a small amount of tissue, in correspondence with the internal hollow body of the fixture.

The retrieved samples were fixed in formalin for 48 h, then dehydrated and embedded in epoxy and processed by means of a cutting grinding appliance. The specimens were cut along their main axis with a diamond disk, then the two halves were mounted on acrylic holders, ground to a thickness of about 35 μm and carefully polished with boron carbide rotating instruments. After a preliminary light microscope examination of the unstained samples, they were carbon-coated and underwent microprobe analysis with the PIXE μ-beam appliance. This technique is based on the proton induced X-ray emission, by means of a very focalized proton beam, that allows to identify and map the elements with an atomic number higher than sodium [17], contained in the sample.

A finely focused proton microbeam is driven across the specimen under investigation, spot by spot analysis is performed by rasting the beam, and analytical information is collected from a spot of submicron diameter. Each specimen, including the fixture and the surrounding tissue, was scanned with fields of about 2.3 mm^2, searching for the presence of metals in the tissues, with a detection limit of about 1 ppm.

Metal spectra were counted for 1 min in several areas on the implant body, on the border bone and at about 0.5 mm from the implant. The counts for the metals in the selected areas were compared with the counts collected on the implant body.

Other sections of each sample were stained with basic fuchsin and methylene blue, and examined with the optical microscope, to compare the histologic findings with the elemental maps obtained with PIXE μ-beam.

Results

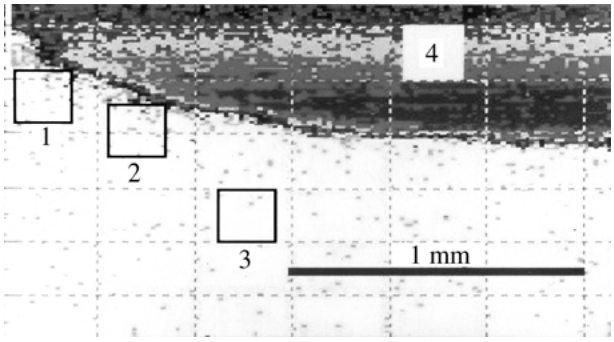
The retrieved implants are shown in Fig. 1(a)–(d), where the attached tissue is also visible. Their composition and surface coating were indicated by the microprobe results and the previously collected informations.

At the optical microscope, implants in Fig. 1(a)–(c) appeared to be osseointegrated, as they were embedded in mature bone, while the specimen in Fig. 1(d) contained inflammatory soft tissue in the implant hollow body.

The legends of the elemental maps show the metal counts per minute on the implant body and on the surrounding bone, and the resulting values are indicated also as percentage of the counts on the implant bulk. This percentage is particularly significant for elements concentration, because the overall counts detected on each sample may vary, depending on slice thickness and its surface morphology.

The most interesting findings were obtained from samples 1–3, as no trace of metal was found in the soft tissue inside the cylinder in sample 4.

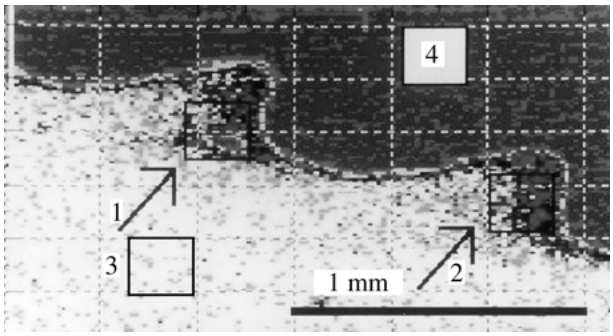
In samples 1–2, the micro beam elemental maps



Total scanned area
1.2 × 2 mm
Scanned areas 1–4 200 × 200 μm

	Counts/min for Ti	% of counts on the implant
Mean	132	
Standard deviation	194.87	
Border bone 1	60	14.22
Border bone 2	44	10.43
Bone 3	2	0.47
Implant bulk 4	422	100

Figure 2 Map for titanium, showing small traces of metal in the peri-implant tissue (lower part of the picture). Indeed, in areas 1–2, counts for titanium are between 10% and 14% compared to the counts for the implant body (upper part). On the contrary, the microprobe indicates, in area 3, at about 0.5 mm from the implant, only 0.47% of the counts in the implant body. This last value is to be considered as negligible, as it is below the precision limit of the microprobe.



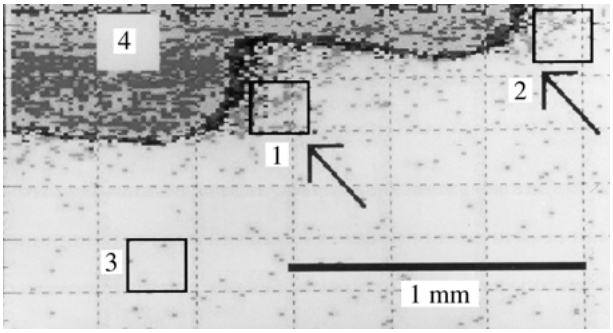
Total scanned area
1.2 × 2 mm
Scanned areas 1–4 200 × 200 μm

	Counts/min for Ti	% of counts on the implant
Mean	574.75	
Standard deviation	346.45	
Border bone 1	691	87.03
Border bone 2	755	95.09
Bone 3	59	7.43
Implant bulk 4	794	100

Figure 3 CP titanium screw in the upper part of the picture. The map for Ti shows accumulation in areas 1–2 (arrows), where counts for Ti in the unit of time are very close to those on the implant. In area 3, at about 0.5 mm from the implant border, counts for Ti decrease to 7.43%.

showed a sharp implant–bone interface, with no tendency of titanium to leak into the bone (Fig. 2), at least in amounts exceeding the detection limit of the micro-beam appliance.

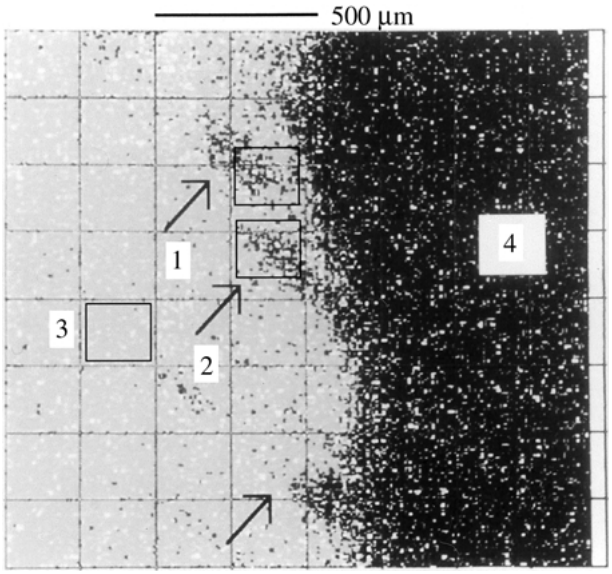
However, areas of Ti deposition into the tissues were occasionally detected either in correspondence with the deepest parts of the threads in the screw implant (Figs. 3 and 4), or in the bone surrounding the unthreaded fixture



Total scanned area
1.2 × 2 mm
Scanned areas 1–4 200 × 200 μm

	Counts/min for Ti	% of counts on the implant
Mean	165.5	
Standard deviation	159.29	
Border bone 1	155	39.64
Border bone 2	90	23.02
Bone 3	26	6.65
Implant bulk 4	391	100

Figure 4 Ti–Al–V titanium screw (up) with related titanium map, showing accumulation of Ti in areas 1–2, while in area 3, at less than 1 mm from the implant, the counts decrease.



Total scanned area
1.5 × 1.5 mm
Scanned areas 1–4 200 × 200 μm

	Counts/min for Ti	% of counts on the implant
Mean	3792	
Standard deviation	3357.09	
Border bone 1	3127	36.89
Border bone 2	3074	36.26
Bone 3	490	5.78
Implant bulk 4	8477	100

Figure 5 Bone (left) – titanium (right) interface in the unthreaded cylindrical plasma-spray coated implant. The map size is 1.5 × 1.5 mm. Titanium deposits are detectable in the bone embedding the implant (arrows), as counts in areas 1–2 indicate.

(Fig. 5). These deposits of Ti were found in about 5% of the bone–implant interface of samples 1–2.

The histologic pictures did not exactly match the microprobe maps for metals (Figs. 6 and 7), as tissue pigmentation, that might suggest metal deposits, was

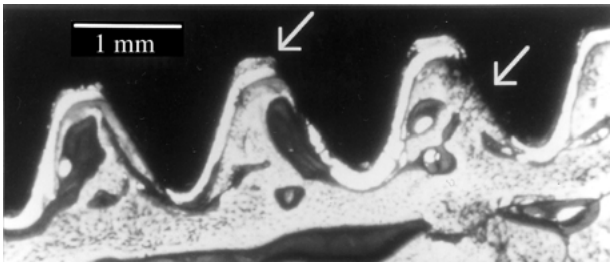


Figure 6 Histologic picture corresponding to Fig. 3. Basic fuchsin and toluidine blue. The arrows point to the fields where Ti accumulation was found by the microprobe, but pigmentation, suggesting the presence of metal, can be seen only at the site pointed by the right arrow.

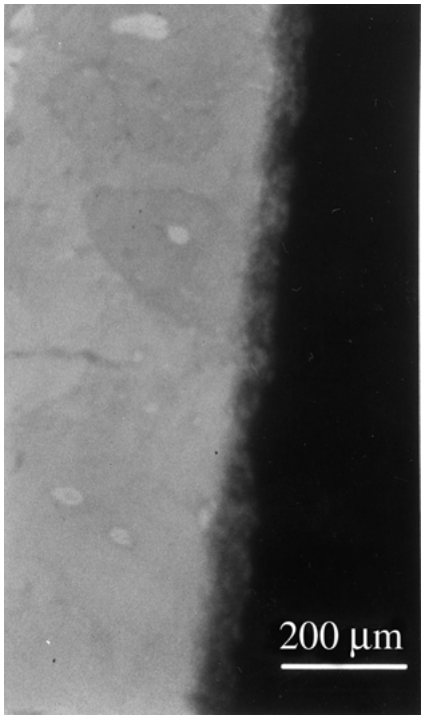


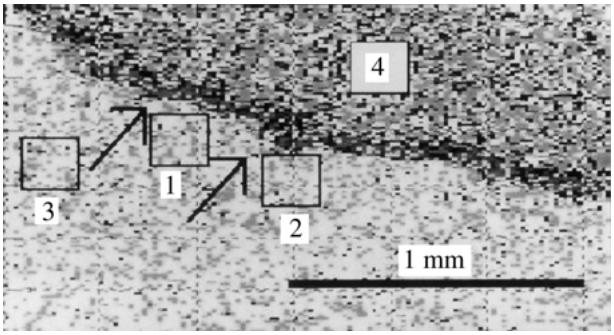
Figure 7 Histologic picture corresponding to the map in Fig. 5. Basic and toluidine blue. Mature bone, with haversian canals, faces the implant. The interface is faded, because of the overlapping of the cutting-edge metal and bone. In spite of the microprobe results, there is no optical evidence of titanium in the bone.

occasionally found in areas where the microprobe indicated metal accumulation (Fig. 6), while similar deposits were not evident at the optical microscope in other samples (Fig. 7).

Sample 3 was made of a Ti–Al–V alloy, and aluminum demonstrated quite a different pattern, as it appeared to leak widely into the surrounding tissues, originating maps where aluminum counts were amply diffused into the peri-implant tissues (Figs. 8–11), while vanadium was not detected around the implant.

Discussion and conclusions

Aluminum, when present in the implant, showed an important uniform leakage into the surrounding bone, in spite of its low percentage in the Ti–Al–V alloys, usually about 5–6%. Some previous *in vitro* studies proved that ion dissolution from Ti–Al–V alloys may be high, especially in orthopedic prostheses with roughened surfaces, and this phenomenon may be reduced, but not



Total scanned area
1.5 × 1.5 mm
Scanned areas 1–4 200 × 200 µm

	Counts/min for Ti	% of counts on the implant
Mean	130.25	
Standard deviation	123.46	
Border bone 1	96	31.17
Border bone 2	95	30.84
Bone 3	22	7.14
Implant bulk 4	308	100

Figure 8 Same sample of Fig. 4 (implant up), with the aluminum map showing diffusion of the metal into the peri-implant tissues. Aluminum also concentrates on the implant surface (arrows).

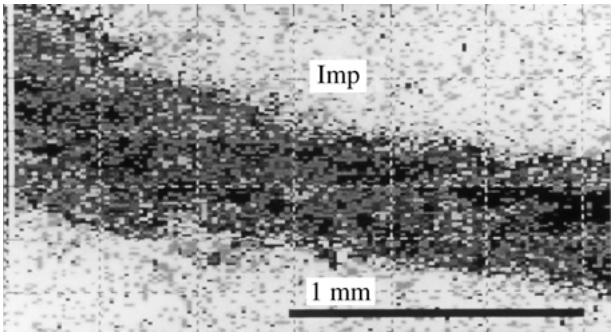


Figure 9 Same sample as in Fig. 4: map of calcium and aluminum, showing the implant facing a bundle of bone.

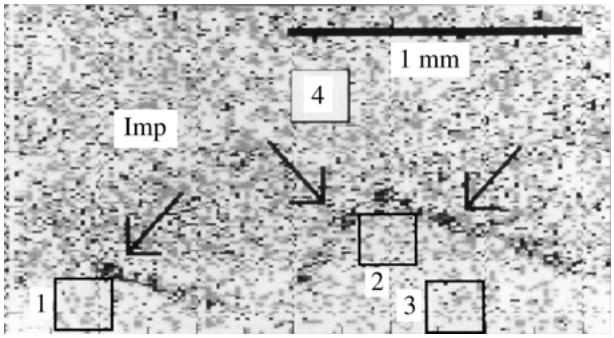
completely avoided, with aging and some surface coatings [30]. The present findings suggest that the presence of aluminum should be avoided in the composition of dental implants, unless a proper coating or surface treatment is applied, that guarantees its chemical inertness.

Indeed, while a possible toxicity of titanium, with evident clinical adverse effects, has yet to be proved, aluminum is to be considered dangerous for the organism, for its well-known neurological and hematological adverse effects [31, 32].

It was not possible to ascertain the brand of the Ti–Al–V implant investigated in this study, but very likely it was handicrafted; on the other hand, the patient had been wearing the screw for 7 years without any local or general disturbance, until the fixture broke due to fatigue, because it served as abutment for an overdenture.

Unlike aluminum, titanium was not found to leak uniformly around the implants, but amounts of Ti were occasionally found in the peri-implant tissues.

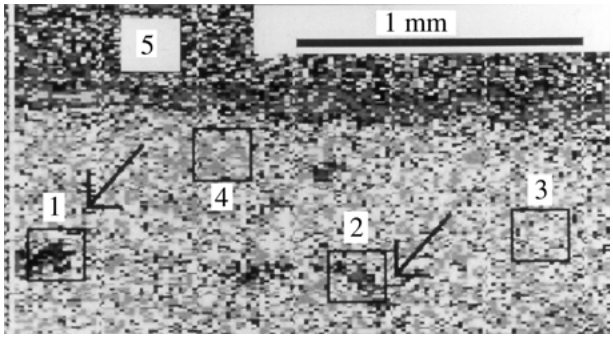
Very likely, these findings may be due to mechanical wear, mainly during the surgical insertion, as underlined by Weingart [23] who inserted titanium implants in dogs



Total scanned area
1.5 × 1.5 mm
Scanned areas 1–4 200 × 200 μm

	Counts/min for Ti	% of counts on the implant
Mean	86	
Standard deviation	12.08	
Border bone 1	81	100
Border bone 2	104	128.4
Bone 3	78	96.3
Implant bulk 4	81	100

Figure 10 Another map for aluminum, showing diffusion of metal in the peri-implant tissues. Counts for aluminum are nearly the same as in the implant body, bordering bone and bone at about 0.5 mm from the implant (up). Aluminum concentrates at the implant surface, making the implant threads visible (arrows).



Total scanned area
1.5 × 1.5 mm
Scanned areas 1–4 200 × 200 μm

	Counts/min for Ti	% of counts on the implant
Mean	521.8	
Standard deviation	226.68	
bone 1	628	75.66
bone 2	551	66.39
bone 3	304	36.63
border bone 4	296	36.66
Implant bulk 4	830	100

Figure 11 Map for titanium and aluminum, with uniform leakage of aluminum and peri-implant deposits of the metal (arrows).

and found similar amounts of metal in the tissues, suggesting a mechanical detachment of the surface layer due to attrition during the surgical screwing. Indeed, titanium has a high attrition coefficient. Metal particles detached from orthopedic prostheses because of wear were also found in the tissues and lymph nodes of patients who had worn hip and knee prostheses for years [33].

The fact that similar masses of titanium could be detected after years from the implant insertion, as in the present study, where most implants had been inserted 6 to 9 years before, may be considered as a further proof of the very low tendency of titanium to diffuse into the body fluids.

Titanium may release ions in solutions, as pointed out by some researches [34], who found that an *in vitro* metal release is induced by macrophages; however, no evidence of such a diffusion *in vivo* has been found yet, and most investigations, in which peri-implant titanium was found, report only occasional local accumulation of the metal, without apparent adverse effects.

However, while vanadium seems to be very inert, and was not found in the tissues, the possible presence of aluminum in the implant is to be considered unadvisable.

The results of this study suggest that titanium may be considered as biologically safe, as its presence in the peri-implant tissues is unconstant, very likely due to localized micro detachments, producing small heaps, that tend to stay locally and may be detected after years, indicating a very low capability of diffusion at distance. However, if clear important adverse effects of titanium are found in the future, more attention will be required in the use of surgical prostheses built with this metal.

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