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Commercially pure titanium and Ti6Al4V implants with and without nitrogen-ion implantation: surface characterization and quantitative studies in rabbit cortical bone

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Nitrogen-ion implantation is a surface modification of interest for biomedical materials. In this study screw-shaped commercially pure titanium and Ti6Al4V alloy implants were nitrogen-ion implanted and analysed by scanning electron microscopy (SEM) and scanning Auger electron spectroscopy (AES). The surface topography was essentially similar for treated and non-treated samples. AES survey spectra showed an incorporation of nitrogen into the surface of the nitrogen-ion treated commercially pure titanium and Ti6Al4V implants, as judged by the ratio between the intensities of the peaks at 390 and 420 eV. AES depth profiles revealed a similar oxide thickness (7.5 nm) for the nitrogen-ion implanted and the non-treated samples. In nitrogen-ion implanted screws nitrogen was detected up to depths of about 150 nm below the surface, with a maximum nitrogen concentration at about 50 nm. Light microscopic examination of the 10 μm-thick ground sections 3 months after the insertion of nitrogen-ion implanted and non-treated commercially pure titanium and Ti6Al4V screws in the proximal tibial methaphysis of rabbits showed that bone filled a large portion of the area within the threads. A fibrous capsule was not observed. Light microscopic morphometry did not reveal any statistically significant differences in bone–metal contact or bone area within the threads of nitrogen-ion treated and non-treated implants. The "mirror-image" analysis showed that for all implants examined significantly more bone was present immediately outside than inside the threads. The results from this study indicate that nitrogen-ion implanted, screw-shaped, commercially pure titanium and Ti6Al4V implants heal as well in cortical bone as non-treated samples.

1. Introduction

The mechanism behind aseptic loosening of arthroplasties is not fully understood. Several studies have shown that, irrespective of cement-fixation or not, inflammatory and immunocompetent cells, including macrophages, lymphocytes and plasma cells are located in the border between metal–cement and fibrous tissue–bone [1–3]. Inflammatory cells have been detected around failed (loose) implants [1] as well as around non-infected, röntgenographically or clinically stable implants [4].

A black staining (pigmentation) and titanium have been demonstrated around failed cemented and non-cemented titanium alloy prostheses [5, 6]. The findings of particulate titanium, macrophages and T-lymphocytes in the fibrous tissue around such failed hip replacements [3] indicate that the outcome of the interaction between wear particles and cells in the implant–tissue interface may be crucial for the stability and long-term function of the prosthesis.

One strategy to improve the longevity of prostheses is therefore to improve the wear properties of the implant components. Ion implantation, a method of increasing the wear and corrosion resistance of metal alloys [7–9], has been used in clinical applications to improve the wear characteristics [8, 9]. This technique has recently also been shown to improve the wear and friction properties of polymers when applied to ultra high-molecular weight polyethylene [10]. However, before new surface treatment techniques of biomedical materials are introduced the tissue response to such surface-modified implants must be evaluated. In previous experiments we studied the biological response of soft tissues to nitrogen-ion implanted titanium [11].

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The aim of the present study was to examine the bone tissue reactions to screw-shaped commercially pure titanium and Ti6Al4V implants with and without nitrogen-ion treated surfaces, after being inserted in the rabbit tibial methaphysis for 3 months.

2. Materials and methods

2.1. Animals and anaesthesia

Seven adult (average age 10 months) New Zealand White male rabbits were used. The rabbits were anaesthetized in conjunction with surgery with intramuscular injections of fentanyl and fluanizon (Hypnorm Vet., Janssen Farmaceutica, Belgium; 0.5 ml kg^{-1} body weight) and intraperitoneal injections of diazepam (Valium, Roche, France; 2.5 mg per rabbit). Local anaesthesia with 1.0 ml 5% lidocaine (Xylocaine, Astra, Sweden) was administered to the proximal tibia, where the implants were to be installed under aseptic conditions. Before surgery the shaved skin of the rabbit legs were washed with a mixture of iodine and 70% ethanol. On the day when they were killed the rabbits were anaesthetized as described above and the tissues were fixed by perfusion.

2.2. Implants and surgical technique

Screw-shaped implants were made from rods of commercially pure titanium (99.75% purity; grade 1) and the alloy Ti6Al4V (grade 5). They were manually manufactured by machining to an outer diameter of 3.5 mm, a core diameter of 2.9 mm and a total length of 8 mm. With a pitch-height of 0.6 mm, 4 mm were threaded. The proximal 4 mm of the implants were square-headed. After an ultrasonic cleaning procedure in trichlorethylene and absolute ethanol half the amount of the commercially pure titanium and the same number of Ti6Al4V implants were ion-implanted with nitrogen to a dose of $5,7 \times 10^{17}$ nitrogen ions cm^{-2}. The ion energy was 80 keV and the temperature reached approximately 220 °C at the end of the ion implantation, due to beam heating (Harwell Laboratory, Oxfordshire, UK). Before insertion in the rabbit legs the implants were again cleaned as described above and finally sterilized in an autoclave.

A gentle surgical technique, involving cutting of the fasciae in separate layers, drilling of bone at low rotary drill speeds using sequential drillbits (surgical steel drills, Ash Drills, UK) and hand-tapping (tap M 3.5 SKF, Sweden) was used. Profuse cooling with saline was used during the bone preparation. The implant insertion site was the proximal tibial methaphysis of both legs. Each rabbit had four implants inserted, two in each leg 5 mm apart. In the right leg the proximal implant was made of commercially pure titanium and the distal implant was nitrogen-ion-treated commercially pure titanium. In the left leg the proximal implant was made of Ti6Al4V and the distal nitrogen-ion treated Ti6Al4V. Twenty-eight implants, seven of each material, were inserted and allowed to penetrate the first cortical layer, never entering the opposite site. The fascia and skin were sutured in separate layers with Vicryl 5-0 and Ethicon 3-0 silk. No external bandage was applied, and immediately after surgery the rabbits were allowed to bear full weight.

2.3. Implant characterization

The surfaces of one nitrogen-ion implanted and one non-implanted (control) sample of each of the two materials were characterized after sterilization. The surface topography was examined with an SEM (Jeol JSM-T-300, Japan). Scanning AES (Perkin–Elmer PHI 600, USA) was used to analyse the surface chemical composition. AES survey spectra (30–1630 eV) were recorded from three different spots (diameter 100 μm) on each sample. The relative concentrations of the detected elements were calculated from the peak heights in the differentiated spectra, after correction by elemental sensitivity factors [12]. Depth profiles were measured at one point (diameter 10 μm) on each sample in order to estimate surface oxide and nitrogen implantation layer thicknesses. A primary electron beam energy of 5 keV and current of 1.0 μA were used in all AES analyses. For sputtering, 4 keV argon ions were used at a sputtering rate of 50 nm min^{-1}, as calibrated for TiO_2. The pressure in the analysis chamber was 1.3×10^{-7} Pa during all AES analysis.

2.4. Preparations of specimens and histomorphometry

On the day when they were killed, i.e. 3 months after insertion of the implants, the rabbits were anaesthetized and the tissues were fixed by perfusion via the left heart ventricle with 2.5% glutaraldehyde in 0.05 M sodium cacodylate, pH 7.4. The implants with surrounding tissue were left in glutaraldehyde overnight and then postfixed in 1% OsO_4 for 2 h. The samples were then dehydrated and finally embedded in acrylic resin (LR White, Polaron Equipment Ltd, Watford, UK). The cutting (of the long axis of the implant) and the grinding of the section (to a thickness of about 10 μm) were carried out using Exakt sawing and grinding equipment. The sections were stained with toluidine blue [13] and analysed by light microscopy in a Leitz Aristoplan. Histomorphometry was performed with a computer-based system (Leitz Microvid equipment connected to an IBM XT computer). All measurements were performed "directly in the microscope", i.e. in the eyepiece of the microscope, using an objective of × 10 and a zoom of × 2.5 when higher magnification was needed. The histomorphometry involved calculations of the total bone–metal contact (around the entire threaded implant), the bone–metal contact in the three best consecutive threads (on each side of the sectioned implant) in the cortical passage, the total bone area and the bone area in the three best consecutive threads. The "mirror-image" analysis was used to compare the bone area occupying the "inside" of a thread to the bone area immediately "outside" the same thread [14].

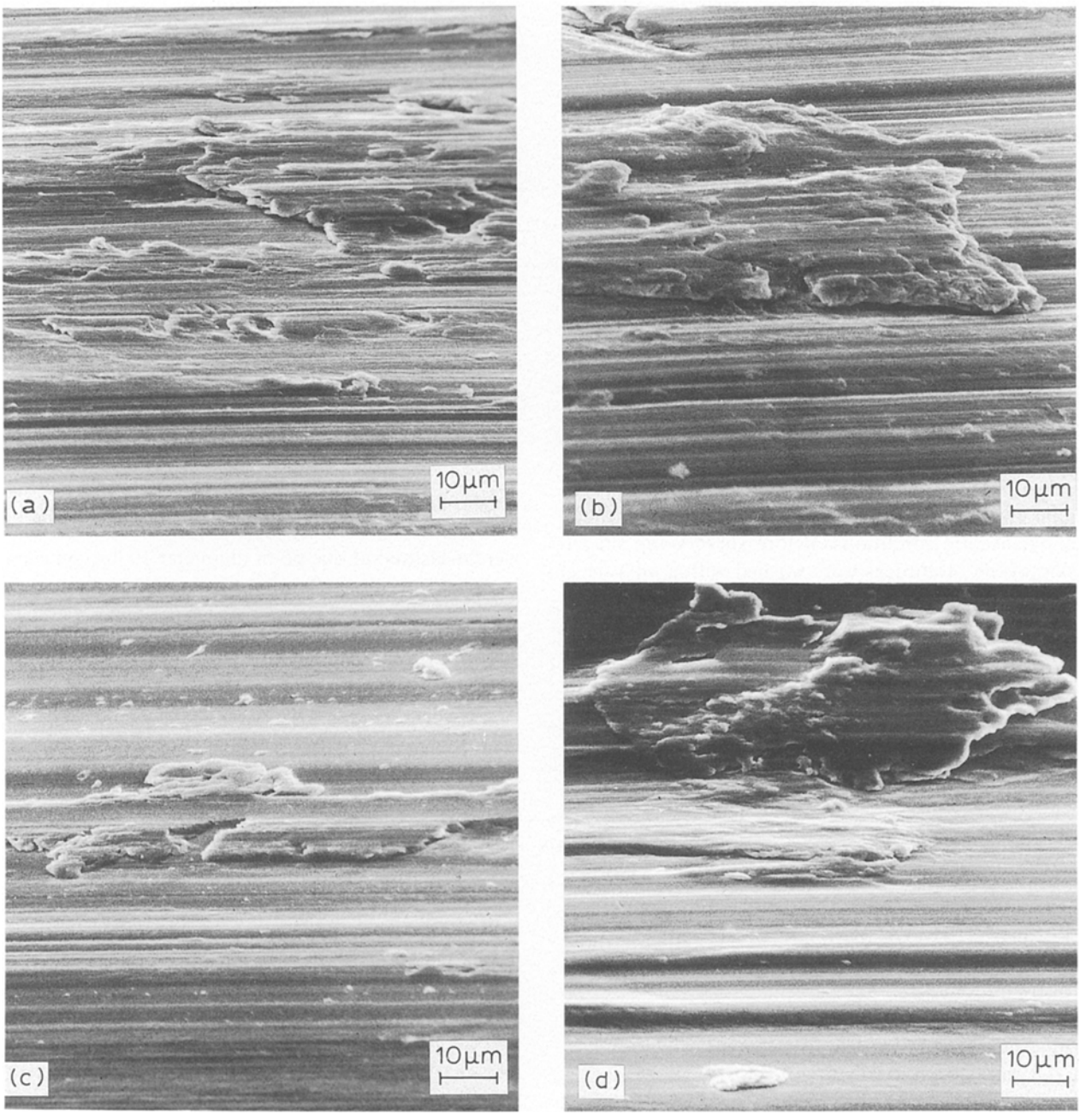

Figure 1 SEM micrographs of: (a) untreated commercially pure titanium implant, (b) nitrogen-ion treated commercially pure titanium implant, (c) untreated Ti6Al4V implant surface and (d) nitrogen-ion treated Ti6Al4V implant surface.

2.5. Statistics

For statistical calculations the Wilcoxon signed-rank test was used.

3. Results

3.1. Implant surface topography

SEM revealed that the implant surfaces had an appearance typical of machined Ti, consisting of microgrooves, small pits and elevated areas (of approximate dimensions 10 μm or smaller, Figs 1–4).

3.2. Implant surface composition

AES survey spectra were dominated by strong Ti and O signals, representative of a Ti surface oxide. Strongly varying C peaks were always detected, and often also minor traces of Al, Ca, Si, Na, P, S and Cl. The elements detected and their relative concentrations are summarized in Table I. The concentrations varied considerably, both between different points on each individual sample and between different samples, but no major systematic differences could be established. The only difference between the Ti and Ti6Al4V materials was that a small high-energy (approximately 1400 eV) Al peak was detected in most of the points analysed on the Ti6Al4V samples, but not on the commercially pure Ti.

Nitrogen is not listed in Table I, since the only available nitrogen Auger peak overlaps with the Ti peak at about 390 eV. However, a qualitative estimate of the amount of N present at the surface can be obtained from the ratio between the intensities of the peaks at 390 eV (Ti + any N) and 420 eV (Ti only)

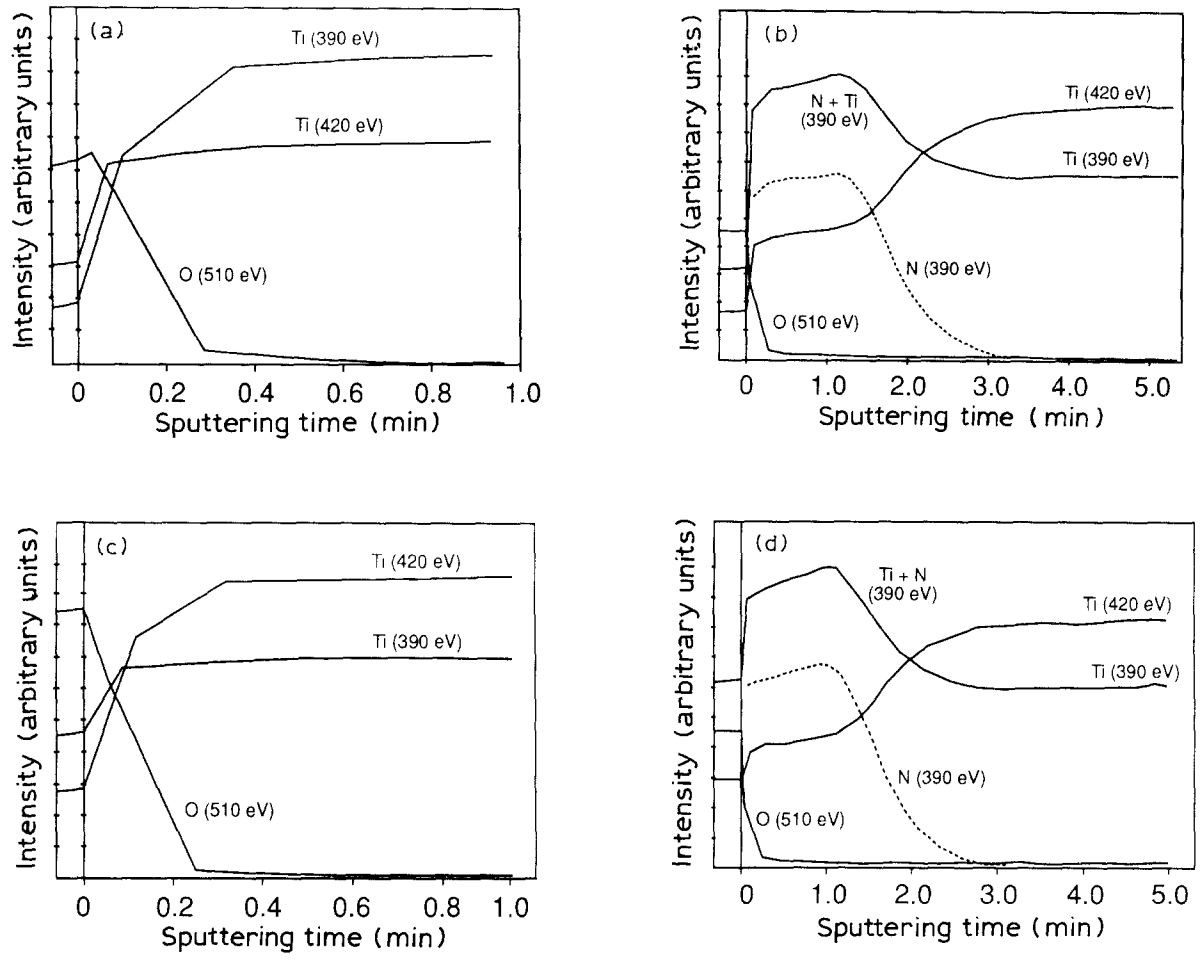

Figure 2 AES depth profiles for the O(510 eV), Ti(390 eV) and Ti(420 eV) peaks. (a) non-implanted/untreated commercially pure titanium, (b) nitrogen ion-implanted commercially pure titanium, (c) non-implanted/untreated Ti6Al4V and (d) nitrogen-ion implanted Ti6Al4V. The broken curves for the ion-implanted samples show the nitrogen profiles.

[15, 16]

$$R = I(390)/I(420)$$

As shown in Table I, the two ion-implanted samples exhibited significantly higher R-values in all points analysed, indicating a higher N concentration on these surfaces.

3.3. Depth profiles

Fig. 5 shows the AES depth profiles measured from the four samples (note the different timescales between the ion-implanted and non-implanted samples). The profiles for the two non-implanted (control) samples had a similar appearance (Fig. 5a and b). The oxygen signal decreased from a maximum level at the outermost surface, and reached a minimum after < 1 min sputtering. This sharp decrease was accompanied by an increase in the two Ti signals as the bulk metal was reached. The depth at which the O signal has decreased to 50% of its maximum value gives an estimate of the oxide thickness. For the two control samples this occurred after approximately 0.15 min, corresponding to a depth of 7.5 nm.

Similar oxide thicknesses were observed for the two ion-implanted samples (Fig. 5c and d). However, the 390 eV peak (corresponding to the overlapping Ti and N peaks) showed a totally different behaviour, with an increased value up to approximately 3 min sputtering (corresponding to about 150 nm) for both ion-implanted samples. This type of profile reflects the presence of significant amounts of subsurface nitrogen in the ion-implanted layer. The qualitative N profiles (derived according to the procedure described by Lausmaa *et al.* [15]) for the two ion-implanted samples is shown by the broken lines. The N depth distributions show approximately the expected Gaussian-like shape. The maximum value for R is 2.1 and occurs at a depth of approximately 50 nm. By comparison with published AES data for Ti nitrides [17], this corresponds to TiN_x ≈ 0.8.

3.4. Light microscopy

Light microscopy revealed continuing bone remodelling within the threads of the implants. In general, a large proportion of the area within the thread was occupied by bone. Bone was observed in direct contact with the implant surface. A periosteal and an endosteal thickening of the bone was demonstrated in the majority of the sections. In areas without bone, macrophages and multinuclear giant cells were often observed in contact with the implant surface. No differences in the general morphology was noted between the implants. On the bottom part of the implants (facing the marrow) a fibrous tissue was

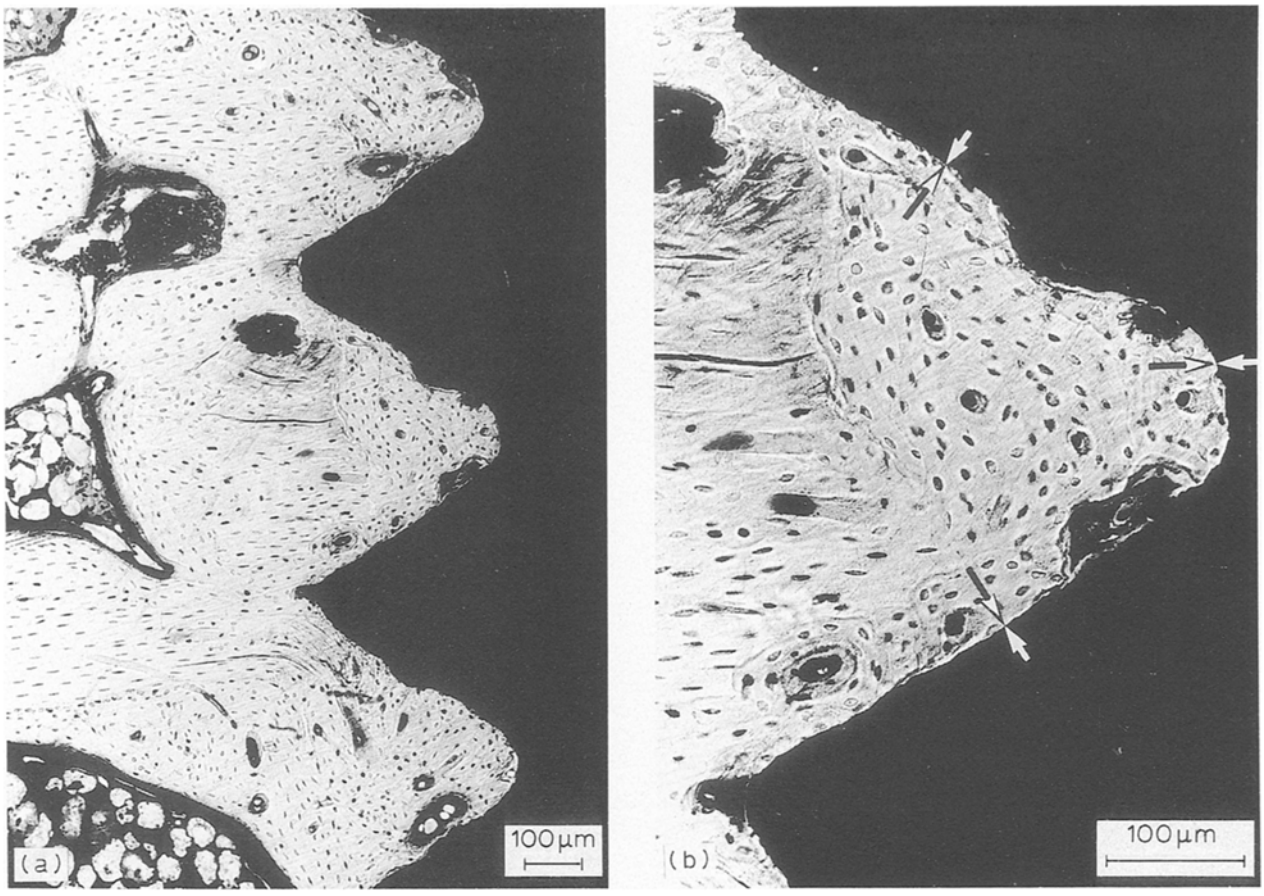

Figure 3 (a) Survey light micrograph of a ground section from an untreated commercially pure titanium implant. A thickening of the bone is observed at the level of the endosteum (bottom) (b) At higher magnification mature bone is observed in tissue in direct contact with the untreated commercially pure titanium implant surface (arrows).

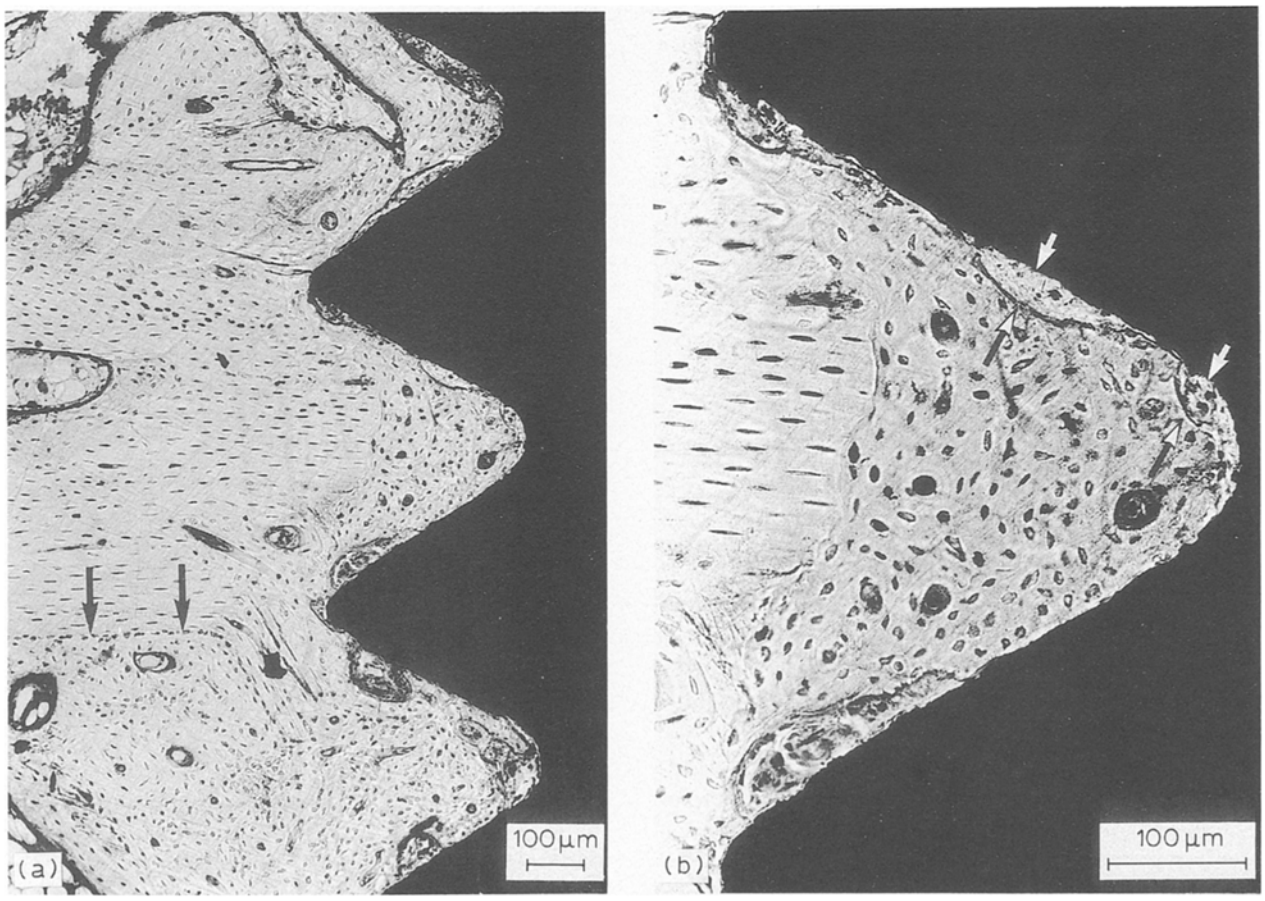

Figure 4 (a) Survey light micrograph of a ground section from a nitrogen-ion treated commercially pure titanium implant The area beneath the endosteum (arrows) consists of compact bone which extends into the threads. (b) Bone tissue is in direct contact with the nitrogen-ion treated commercially pure titanium implant surface. Areas with a soft tissue interface are indicated (arrows).

TABLE 1 Relative concentrations (at %) of elements and *R*-ratios measured by AES survey analyses of the four different implants *R*-values represent the measured ratio between the intensity of the Ti + N(390 eV) and Ti (420 eV) peaks

		Commercially pure Ti, non-implanted			Commercially pure Ti, ion-implanted			Ti6Al4V, non-implanted			Ti6Al4V, ion-implanted		
Peak (eV)	Sensitivity factor	Point 1	Point 2	Point 3	Point 1	Point 2	Point 3	Point 1	Point 2	Point 3	Point 1	Point 2	Point 3
Ti (420)	0.33	23 4	22.2	21.5	15.5	12.8	18.6	13.7	21.2	13.0	28.9	19 2	33.8
O (510)	0 41	56 4	55.2	68 2	42 7	42.8	52.2	42.5	51.0	41 1	48 6	45.1	53 5
C (273)	0 14	15.3	16 5	4.5	31.3	32.6	21 3	40.4	18 0	39.0	15.2	28.7	6 8
Al (1395)	0 07								2.2	0 9	1.8	1 1	2.9
Ca (292)	0.31	3.8	3 4	3.5	5 2	8.4	3.9	1 4	1.6	1.5	1.7	2.5	1.9
P (115)	0.45	0.5								0 2			
S (150)	0.75	0.6	0 5	0.9	0.7	1.1	0.9	0.5	1 2	0.7	0.4	0.3	
Cl (180)	1 10			0.9	0 3	0.5		0.2	0 1	0.8			
Na (990)	0.25				1.9	1 7	3.2				0.8	3 1	1.1
Si (90)	0 10		2.1	0.5	2 4			1.3	4.7	2.8	2 5		
R		1.69	1.71	1 63	1.92	1.90	1.87	1.71	1.75	1.67	1.92	2.02	2.11

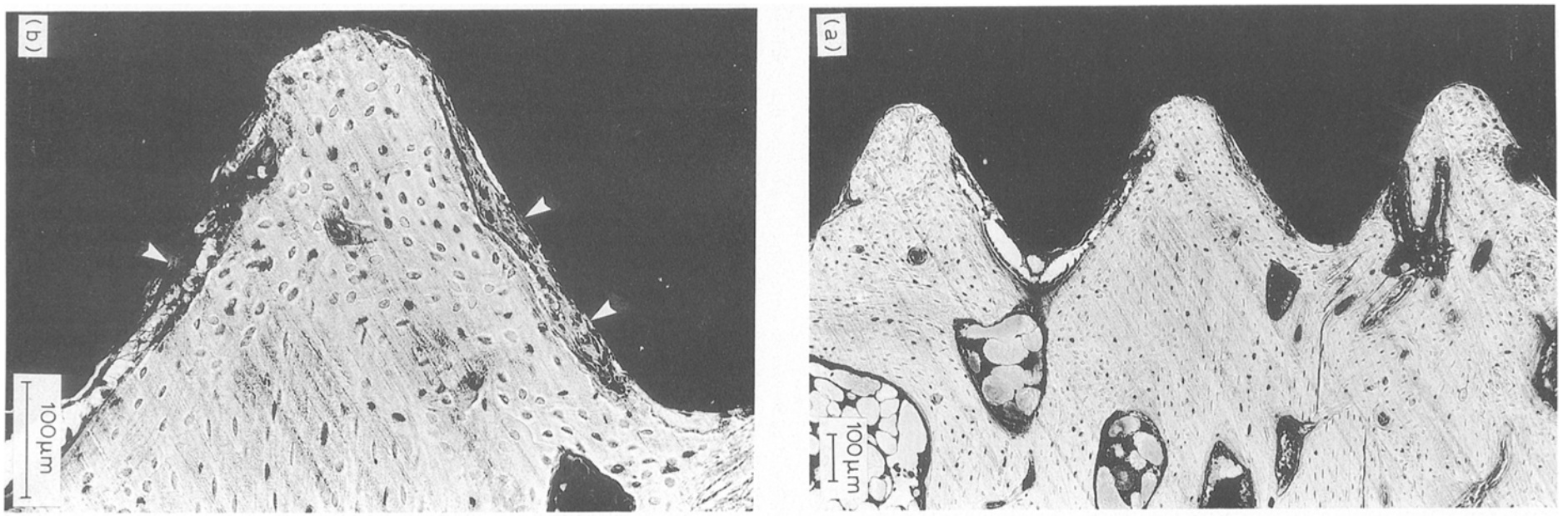

Figure 5 (a) Survey light micrograph of a ground section from an untreated Ti6Al4V implant. (b) Mature bone is in direct contact with the untreated Ti6Al4V implant surface. Areas with a soft tissue interface are indicated (arrowheads).

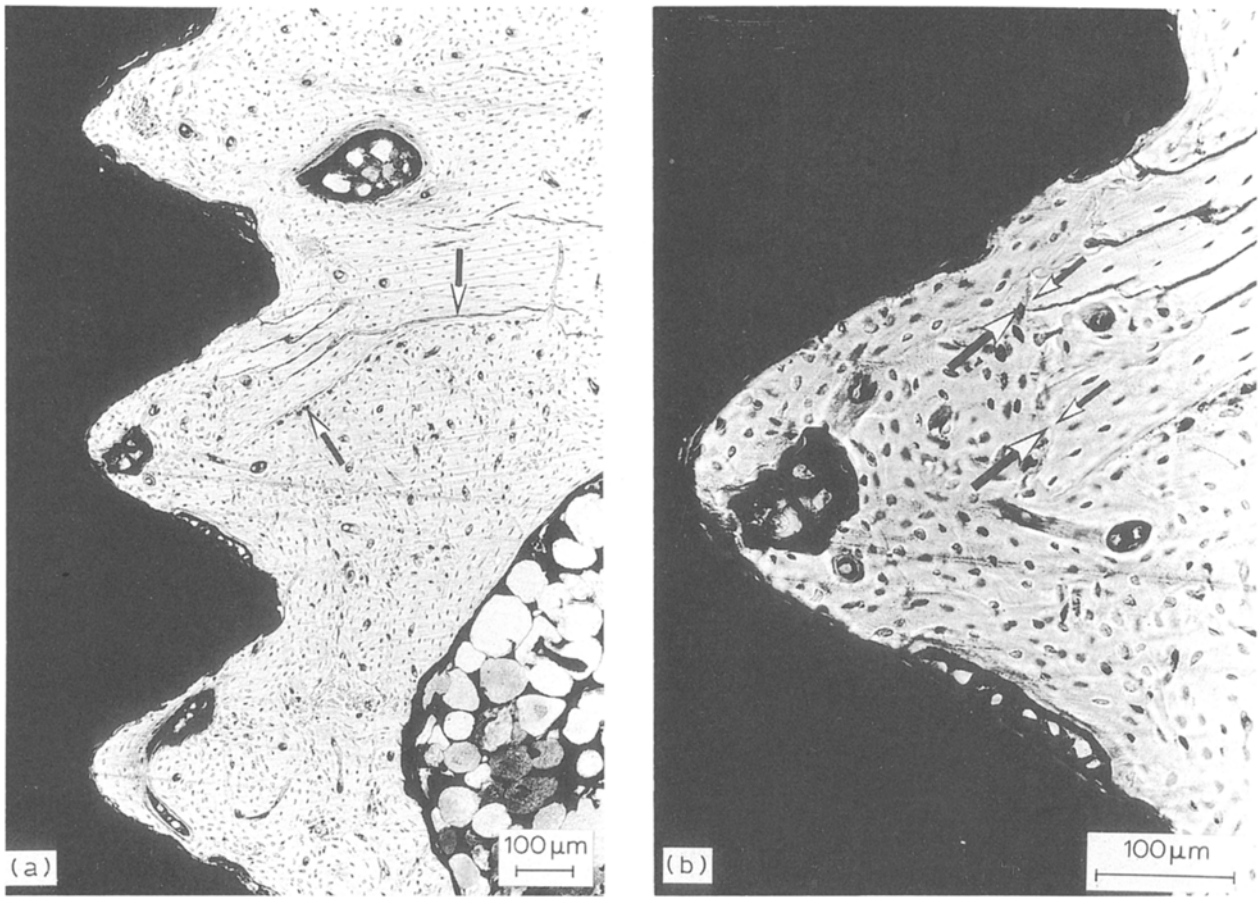

Figure 6 (a) Survey light micrograph of a ground section from a nitrogen-ion treated Ti6Al4V implant. The border between the old endosteal surface and the newly formed bone is indicated (arrows). (b) Mature bone and soft tissue are in direct contact with the nitrogen-ion treated Ti6Al4V implant surface. The border between the old, cut bone and newly formed bone is within the thread (arrows).

detected. The soft tissue consisted mainly of fibroblasts and collagen, and was arranged as a dense capsule separating the metal surface from the marrow cavity.

The mean total bone–metal contact around the entire surface (Table II) were for the untreated commercially pure titanium 26.2% (range 18.1–36.9%) and for the ion-implanted commercially pure titanium implants 24.7% (range 14.5–34.2%; no statistically significant difference; $P = 0.353$). The untreated Ti6Al4V implants demonstrated a mean of 21.2% (range 15.2–35.6%) and around the ion-implanted Ti6Al4V implants a mean of 18.4% (range 12.8–25.8%) was calculated (no statistically significant difference; $P = 0.447$). No statistically significant differences in total bone–metal contact were obtained between the two metals (untreated commercially pure titanium versus untreated Ti6Al4V; $P = 0.076$, and ion-implanted commercially pure titanium versus ion-implanted Ti6Al4V; $P = 0.076$).

The bone-metal contact in the three best consecutive threads (Table III) of the untreated commercially pure titanium implants was 43.6% (range 22.5–54.3%), whereas the ion-implanted commercially pure titanium it was 37.4% (range 28.5–48.9%). The corresponding values for the untreated and nitrogen-ion treated Ti6Al4V implants were 34.6% (range 17.9–56.8%) and 29.5% (range 20.6–36.8%), respectively. No statistically significant differences were observed between the untreated and the ion-implanted commercially pure titanium implants ($P = 0.205$) or between the untreated and the ion-implanted Ti6Al4V implants ($P = 0.353$). No statistically significant differences were obtained between the two metals (untreated commercially pure titanium implants versus untreated Ti6Al4V; $P = 0.108$ and ion-implanted commercially pure titanium implants versus ion-implanted Ti6Al4V; $P = 0.076$).

TABLE II Bone–metal contact (%) around the entire implant [U, untreated (normal) and I, ion-implanted]

	Mean	Median	SEM
U commercially pure Ti	26.1	27.4	2.6
I commercially pure Ti	24.7	26.2	2.6
U TiAlV	21.2	20.5	2.8
I TiAlV	18.4	19.2	1.7

TABLE III Bone–metal contact (%) in the three best consecutive threads in the cortical passage [U, untreated (normal) and I, ion-implanted]

	Mean	Median	SEM
U commercially pure Ti	43.6	48.3	5.3
I commercially pure Ti	37.4	34.4	2.9
U TiAlV	34.6	34.9	4.7
I TiAlV	29.5	30.5	2.3

TABLE IV Bone area (%) around the entire implant [U, untreated (normal) and I, ion-implanted]

	Mean	Median	SEM
U commercially pure Ti	54.2	55.2	2.1
I commercially pure Ti	54.0	55.2	2.2
U TiAlV	53.5	52.2	1.5
I TiAlV	54.1	57.9	4.1

TABLE V Bone area (%) in the three best consecutive threads in the cortical passage [U, untreated (normal) and I, ion-implanted]

	Mean	Median	SEM
U commercially pure Ti	82 9	83.1	1.1
I commercially pure Ti	81.1	80.8	1.3
U TiAlV	82 2	83.1	0.7
I TiAlV	81.2	82.5	1.3

TABLE VI Mirror-image area (%) "inside" and "outside" commercially pure titanium threads [U, untreated (normal) and I, ion-implanted]

	Mean		Median		SEM	
	Inside	Outside	Inside	Outside	Inside	Outside
U commercially pure Ti	83.8	93.5	83.6	93 5	1.1	1 1
I commercially pure Ti	82.1	93.2	81.0	93.7	0 9	1.2

TABLE VII Mirror-image area (%) "inside" and "outside" Ti6Al4V threads [U, untreated (normal) and I, ion-implanted]

	Mean		Median		SEM	
	Inside	Outside	Inside	Outside	Inside	Outside
U Ti6Al4V	80.9	88.0	81.2	88.7	0.9	2.7
I Ti6Al4V	82.0	91 3	82 5	93.9	1.4	2.3

Calculations of the total bone area (Table IV) around the implants revealed similar values in the four different groups: untreated commercially pure titanium 54.2% (range 46.4–61.4%); ion-implanted commercially pure titanium 54.0% (range 47.0–63.8%); untreated Ti6Al4V 53.5% (range 49.7–60.7%); and ion-implanted Ti6Al4V 54.1% (range 38.6–65.5%). There were no statistically significant differences obtained in any of the comparisons among the different groups.

Similarly, the mean percentages of bone area in the three best consecutive threads (Table V) were: untreated commercially pure titanium 82.9% (range 78.4–85.5%), nitrogen-ion treated commercially pure titanium 81.1% (range 76.2–86.0%; no statistically significant differences between these two groups; $P = 0.199$). In the case of the untreated Ti6Al4V implants a mean of 82.2% (range 79.1–83.9%) was demonstrated and for the ion-implanted Ti6Al4V sections a mean of 81.2% (range 77.0–85.1%) was obtained (no statistically significant difference between the two groups; $P = 0.249$). A comparison of the bone area in the three best consecutive threads between untreated commercially pure titanium and untreated Ti6Al4V as well as ion-implanted commercially pure titanium and ion-implanted Ti6Al4V did not reveal any statistically significant differences ($P = 0.433$ and $P = 0.306$, respectively).

The "mirror-image" analysis of the untreated commercially pure titanium cases (Table VI) revealed an "inside" mean value of 83.8% (range 78.4–87.0%) and an "outside" mean value of 93.5% (range 89.9–97.0%; statistically significant difference between the inside–outside values; $P = 0.009$). The percentage bone area "inside" the ion-implanted commercially pure titanium threads revealed a mean of 82.1% (range 77.3–87.1%), whereas the mean "outside" value was 93.2% (range 89.7–96.4%; statistically significant difference between the inside–outside values; $P = 0.009$).

The mean percentage bone area occupying the "inside" of the untreated Ti6Al4V threads (Table VII) was 80.9% (range 77.4–84.2%), whereas the "outside" mean value was 88.0% (range 75.9–96.1%; statistically significant difference; $P = 0.031$). For ion-implanted Ti6Al4V a mean "inside" value of 82.0% (range 74.4–85.8%) and a mean "outside" value of 91.3% (range 79.5–97.0%) were obtained (statistically significant difference; $P = 0.014$).

4. Discussion

The surface analysis results showed many similarities between the analysed samples, but also some differences. A major difference was the higher concentration of nitrogen, both at the surface and up to depths of approximately 150 nm in the ion-implanted materials, compared with in the controls. Based on results from previous X-ray powder spectroscopy studies of similar materials [15], the nitrogen can be assumed to be present in the form of Ti nitride. Minute amounts (< 1%) of Ti nitride have previously been detected at the surfaces of non-ion-implanted Ti and Ti6Al4V materials [16, 18]. However, in analogy with the earlier studies, the present results demonstrate a significantly higher nitrogen level in the ion-implanted materials. From the existing spectroscopic data from

nitrogen-ion implanted Ti and Ti6Al4V, it is not possible to conclude whether the outermost surface consists of a thin TiO_2 surface oxide over the nitrogen-implanted layer or of a mixed oxynitride. In the former case the ion-implanted and non-implanted materials would present essentially similar surfaces (from a purely compositional point of view) to the biological environment, whereas the latter case implies that different chemical conditions are present.

Not unexpectedly, another observation was the presence of Al at the outermost surface and in the oxide layer of the Ti6Al4V samples. This observation corroborates previous findings by Ask *et al.* [18, 19] and suggests a different oxide composition of Ti6Al4V from the almost pure TiO_2 found on commercially pure titanium. Furthermore, the presence of Al in the surface oxide of the Ti6Al4V did not seem to be influenced by the nitrogen-ion implantation.

Most probably, the relatively wide spread in surface composition, both between different points analysed on each individual sample and between the different samples, is mainly an effect of the variations in the carbon levels. The reason for this is that the carbon signal is due to an adsorbed hydrocarbon overlayer, which covers the implant surfaces [15, 16]. Variations in this contamination layer can strongly influence the detected levels of other (underlying) elements. For the same reason it is possible that additional differences in surface characteristics which originally could have existed between the samples used in this study were screened out during the sterilization process. It has previously been pointed out that autoclaving leads to chemical contamination of the surfaces [20]. The surface composition of the present samples is therefore determined to a large extent by the contamination layer formed during the autoclaving process. This makes it difficult to establish any clear differences among the four types of samples. The use of another, "cleaner" sterilization method (e.g. gamma-irradiation) will probably result in surfaces with more pronounced differences in surface characteristics.

The surface topography was essentially similar for the samples (at least within the resolution level of the SEM), and the oxide thicknesses were similar to those previously found by us and others. However, it can be expected that the ion-implantation treatment introduces various microstructural defects into the surface layer of the material. Such effects were not investigated in this study, and would require the use of transmission electron microscopy and X-ray diffraction analysis.

Our light microscopic observations showed similar bone tissue reactions around the materials. Also, the morphometric analysis of the degree of bone–implant contact and the area of bone occupying the inside of the threads did not reveal any statistically significant differences between the materials. In a previous experimental study [11] focused on the number and distribution of macrophages adjacent to nitrogen-ion treated and non-treated pure titanium surfaces in the soft tissue of rats, a similar tissue response was observed around the two materials, with the exception of one time-point (6 weeks) at which a greater number of macrophages were detected close to nitrogen-ion implanted titanium. In the present study, although not statistically verified, the morphometric evaluation, both around the entire implant and in the three best consecutive threads in the cortical region, revealed more bone area and contact for the non-ion-implanted commercially pure titanium and Ti6Al4V implants in comparison with the ion-treated samples. It is therefore important to evaluate whether these minute differences after 3 months healing are observed after longer implantation periods. Since it is possible that differences may exist in terms of cell function on these surfaces, further biological and clinical experiments must be evaluated with additional analytical tools. As discussed above, the possibility that biologically relevant differences in the physical and chemical properties of the tested materials were screened out by the sterilization procedure suggests that additional surface cleaning techniques should be applied before implantation.

In summary, the present study has shown that non-implanted and nitrogen-ion implanted commercially pure titanium and Ti6Al4V have different surface characteristics. In addition to the intrinsic differences between commercially pure titanium and Ti6Al4V, the nitrogen implantation treatment resulted in an incorporation of nitrogen up to depths of approximately 150 nm below the surface. The maximum N concentration corresponded to $TiN_x \approx 0.8$ and occurred at about 50 nm. No subsurface nitrogen was detected in non-implanted samples. After 3 months insertion in rabbit cortical bone of the four materials, no major differences in the degree of bone–implant contact or bone area within the screw threads were observed. Thus, the detected differences in surface characteristics did not influence the tissue response as observed at the light microscopic level after 3 months. Our results indicate that autoclaved nitrogen-ion implanted implants made from commercially pure titanium and Ti6Al4V may heal as well in cortical bone as the corresponding non-implanted materials.

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