JOURNAL OF MATERIALS SCIENCE **36** (2001) 1987 – 1993

Bone response to machined cast titanium implants

S. MOHAMMADI*
Institute of Anatomy and Cell Biology, Göteborg University, Göteborg, Sweden; Department of Orthodontics, Göteborg University, Göteborg, Sweden
E-mail: odosh@odontologi.gu.se

M. ESPOSITO
Institute of Anatomy and Cell Biology, Göteborg University, Göteborg, Sweden

L. WICTORIN
Structural Biomaterials, Institute of Surface Chemistry, Stockholm, Sweden

B.-O. ARONSSON
Group of Applied Physics—Biomedical, University of Geneva, Geneva, Switzerland

P. THOMSEN
Institute of Anatomy and Cell Biology, Göteborg University, Göteborg, Sweden

The aim was to evaluate the bone response to machined cast titanium (Ti) implants. Commercially pure (c.p.) machined Ti implants served as controls. Analyses of the surface composition and topography by Auger electron spectroscopy (AES) and scanning electron microscopy (SEM) revealed no differences comparing the two materials. Cast screw-shaped and identical machined Ti implants were inserted in the tibial metaphysis of 6 rabbits. After 3 and 6 months, the amount of bone within threads and the degree of bone-implant contact were histomorphometrically evaluated. The bone area of cast Ti implants was 45% after 3 months and 62% after 6 months. The corresponding values for machined Ti implants were 51% and 58%, respectively. The total bone-implant contact for cast Ti implants was 19% (25% control implants) after 3 months and 45% (37% for control implants) 6 months after implantation. No statistically significant differences were observed between the two materials at any time interval. The present experimental results indicate that machined cast Ti implants integrate equally well in bone as machined c.p. Ti implants do.

1. Introduction
Cast titanium is increasingly used in restorative dentistry because of its mechanical properties, biocompatibility and economical advantages [1–3]. However, with the exception of 2 clinical reports [4, 5], cast Ti implants have not been used as implant material. The increased hardness of cast Ti, when compared to conventional machined Ti [6–8] could be advantageous in some medical applications. However, the casting procedure of Ti is influenced by several factors, which are crucial for its outcome [8]. Molten Ti is extremely reactive with the elements present in the casting investment [8, 9]. Cast Ti has previously shown some unfavourable properties such as poor surface quality and dimensional inaccuracies [7, 10], as well as the presence of elements, such as P, O, Al, Mg and Si, derived from the mold [8]. In a previous study, porosities, cracks, elevations and depressions were detected on the surface of cast Ti compared to the surface of machined commercially pure (c.p.) Ti implants [11]. Interestingly, the biological response to cast titanium in soft tissues was similar to that of machined c.p. Ti implants, with the exception of considerably more multinuclear giant cells around cast Ti implants [11]. The biological response to cast Ti has never been investigated in bone. The aim of the present study was to evaluate the bone response to cast Ti implants in relation to machined c.p. Ti implants. In order to decrease the contamination derived from the casting procedures, the surface of cast Ti was machined.

2. Materials and methods
2.1. Casting procedure and implants
Test implants were cast from wrought Ti rods (grade II; JMI-125 from JMI-Ti Ltd; diameter 21 mm). Casting was performed in a dental casting machine (Castmatic, Iwatani & Co., Ltd., Osaka, Japan) [8]. The mould was made of Ti grade I (Rema Titan, Dentaurum, Ispringen,

* Author to whom all correspondence should be addressed.

Germany). The mould material was mixed with water and treated according to the recommendations given by the manufacturer. The manufactured cast rods had a diameter of 3 mm and were 4 mm long. Control implants were manufactured by machining of grade I rods (Edstraco AB, Årsta, Sweden).

Implants (diameter 2.5 mm and length 3.5 mm) were threaded from cast and c.p. Ti by machining in a turning lathe. About 0.25 mm of the superficial layer of the cast Ti, rich of impurities, was therefore removed. The top of the implants had a slit to fit a screw-driver during insertion. Prior to implantation, the implants were cleaned ultrasonically in trichloroethylene for 15 minutes and absolute ethanol for 3×10 minutes. The implants were then sterilized by autoclaving.

2.2. Surface implant characterization

The surface elemental composition of two samples of each implant type was analyzed with scanning Auger electron spectroscopy (AES) (Perkin Elmer PHI 660, Eden Praire, USA). Survey spectra (30–1630 eV) were recorded from two points located at the end and four points located in the thread portion on each sample. Depth profiles were measured in one or two points at each sample (Fig. 1). All spectra were taken at 5 keV primary electron energy with an electron beam current of 0.6 μA, electron beam diameter of $\approx$180 μm, and an energy resolution of 0.6%. Relative concentrations (in atomic percent, at%) of the detected elements were calculated from their peak-to-peak values in differentiated spectra after correction with the elemental sensitivity factors [12]. This procedure gives the average concentrations of the detected elements within the probed volume (typically the 3–10 outermost atomic layers), and does neither take into account the depth distribution of the elements, nor chemically induced variations in the sensitivity factors. Therefore, the quoted concentrations should not be regarded as absolute surface concentrations. However, comparison between the different samples can be made, since they were analyzed under identical conditions. Oxide thicknesses were estimated from AES depth profile analyses, sputtering with 2 keV argon ions, rastered over a 2×2 mm^2 area. The oxide thickness was taken as the depth at which the oxygen signal had decreased to half of its intensity at the oxide surface. The sputtering rate was $\approx$35 Å/min, as calibrated for Ta_2O_5, which corresponds to approximately 17 Å/min for TiO_2. The surface topography of machined cast and machined c.p. titanium implants was examined by scanning electron microscopy (Jeol JSM T-300).

2.3. Surgical procedures

Six New Zealand White female rabbits (Alab, Södertälje, Sweden), weighing 4–5 kg, were anesthetized with intra-muscular injections of fluanizole (Hypnorm®, Jensen, Brussels, Belgium, 0.7 mg/kg body weight) and intraperitoneal injections of diazepam (Stesolid®, Dumex, Copenhagen, Denmark, 1.5 ml/kg body weight). Additional Hypnorm was given when needed. Local anaesthesia was induced with 1.0 ml of 5% Xylocain® (Astra, Södertälje, Sweden) at the surgical site. The proximal tibial metaphysis was exposed via a skin incision and a careful subperiosteal dissection was made under sterile conditions. Two holes were drilled (one proximal and one distal, 1 cm apart from one another in each tibia), subsequently enlarged from 1.0 to 2.3 mm, and pretapped under profuse saline irrigation. The implants were installed in level with the cortical bone. Thus, each tibial bone received 2 implants of each type. The periosteum and fascia were sutured by a resorbable suture and the skin by a silk suture. No external bandages were applied. Postoperatively, the animals were given bensylpenicillin (Penovet vet.®, Novo Nordisk Pharma AB, Malmö, Sweden, 300 mg/ml, 20 mg/kg body weight) and analgetics (buprenorphine, Temgesic®, Meda Sverige AB, Göteborg, Sweden, 0.05 mg/kg body weight) as single intramuscular injections. Each rabbit was operated twice. Right tibias were operated first and left tibias, 3 months after. The implantation schedule was determined so that each implant type was inserted equally both in the proximal and distal holes throughout the experiment. Three months after the second operation, the rabbits were sacrified. Therefore, there were 2 follow-up periods of 3 and 6 months.

2.4. Tissue preparation

At the day of sacrifice the animals were anesthetized with an overdose of intra-venous barbiturates (Mebumal®, ACO, Solna, Sweden) and fixed by vital perfusion via the left heart ventricle with 2.5% glutaraldehyde in 0.05 M sodium cacodylate buffer, pH 7.4. The implants were exposed and removed *en bloc* and further fixed by immersion in glutaraldehyde for 24 hours and postfíxed in 2% osmium tetroxide for one hour. After dehydration in a graded series of ethanol, the specimens were embedded in plastic resin (LR White, The London Resin Co Ltd., Hampshire, England). The embedded implants were divided longitudinally by sawing and one half section was ground (Exakt Apparatebau, Norderstedt, Germany) to approximately 10–20 μm [13], stained with 1% toluidine blue and examined in a Nikon Microphot FXA microscope.

2.5. Histomorphometry

The histomorphometrical calculations were performed with a Leitz Microvid equipment connected to an IBM XT computer. The percentage of bone in direct contact with implant and the bone area within threads were calculated for the entire implant surface (i.e. both implant sides were evaluated) with a $\times$ 10 eyepiece (total magnification $\times$ 125). The student paired comparison t-test with a 95% confidence level was used for statistical analysis.

3. Results

3.1. Surface composition and oxide thickness

SEM showed pits, grooves and elevations on both machined cast Ti and machined c.p. Ti implant surfaces. No major qualitative differences in surface roughness could be detected (Fig. 1).

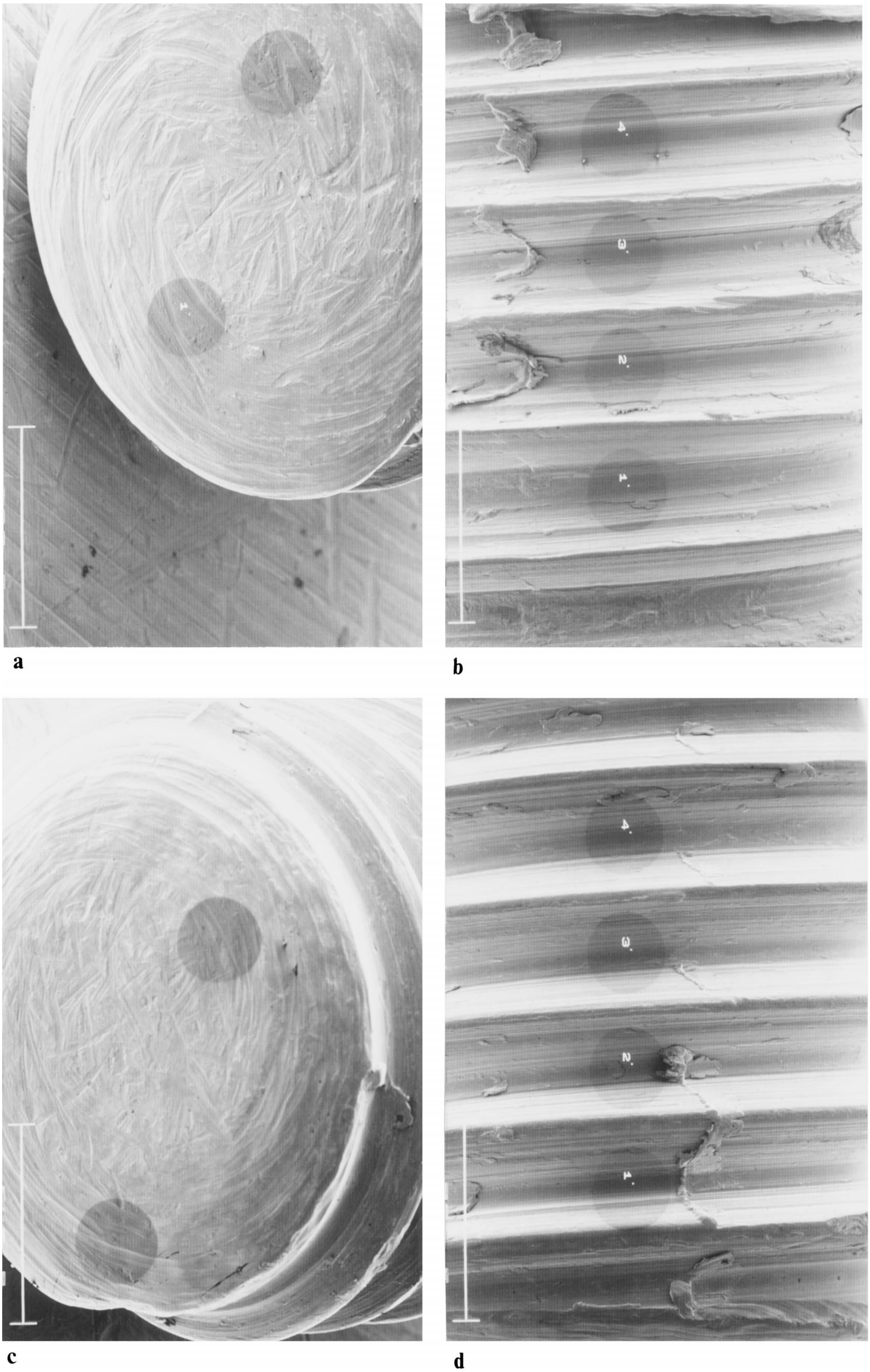

Figure 1 Scanning electron micrographs of machined cast (a and b) as well machined wrought (c and d) Ti. The round dark areas were caused by the electon beam during AES surface elemental composition. Survey spectra were recorded from four points located in the thread portion (b and d) and two points located at the end portion (a and c) of each sample. Note the the grooves, pits and fragments determined by the machining procedure. Bar $=500\ \mu$m.

TABLE I Auger electron spectroscopy of machined cast and wrought Ti screws

sample #	Ti (std)	O (std)	C (std)	Ca (std)	other (std)
1	11.5	37.4	48.2	1.6	1.2
	(1.1)	(2.8)	(3)	(0.5)	(1)
2	11.4	37.3	47.6	2.3	1.4
	(1.6)	(4.2)	(4.8)	(0.3)	(0.9)
3	11.4	37.4	47.9	1.8	1.5
	(1.4)	(4.6)	(4.4)	(0.3)	(1.8)
4	11.6	38.1	46.5	2.8	1
	(1.1)	(4.2)	(4.3)	(0.3)	(0.9)

In the analyzed volume portion of the surface (with a beam diameter $\approx$180 μm and an average electron escape depth $\approx$2.5 nm), the main elements are Ti and O, in the form of TiO_2, and C, mainly as hydrocarbons. Ca as well as S, Na and Cl (denoted as "other" elements in the table) are occasionally present in the analyzed points. Together with the hydrocarbons they are adsorbed on the oxide surface. Samples #1 and #2 are machined from cast Ti, whereas samples #3 and #4 are machined from Ti rods.

The relative elemental concentrations detected in AES survey spectra are presented in Table I. All samples had a relatively similar surface composition, dominated by strong Ti, O and C signals, independent of preparation. Survey spectra (not shown) revealed that implant surfaces were covered by an expected contamination layer. The predominant surface contaminant was C, most probably from adsorbed hydrocarbons originating from rinsing solvents, the autoclaving and air exposure. The C contamination levels are typical for autoclaved samples and did not vary much between the different samples, however, a higher concentration of C was observed on the bottom of the screws than on the threaded portion. Small amounts of Fe ($\leq$2.2 at%) were detected on the bottom and trace amounts (up to a few at%) of Ca, S, B, Na and Cl were detected on all surfaces examined (Table I).

The two types of samples had thin oxides (2–5 nm), with no significant difference between them. The shapes of the AES Ti_{LMV} peak indicated that the surface oxides had a TiO_2-like stoichiometry [14]. However, the O/Ti_{418} eV peak height ratio was $\sim$3.3 for all samples. These high O/Ti ratios are most likely due to excess O bound to elements other than Ti, such as hydroxyl groups, adsorbed water, and carbon oxides [15].

3.2. Tissue organization and morphometry

After 3 as well as 6 months, macroscopical observations showed that all implants, irrespective of type, were covered by bone. Light microscopic observations on ground sections showed that both types of material were surrounded by and in contact with mature cortical bone (Fig. 2). At both observations periods, histomorphometry revealed that the proximal threads (1–4 threads of total 7 threads per implant) were occupied by more bone and had a greater degree of bone contact than the 3 distal threads projecting into the bone marrow cavity. Bone occupied 45% ($\pm$4.5 = standard error of the mean; $n = 6$) of the area within the threads of machined cast Ti implants, 3 months after implantation (Fig. 3a). The bone area increased to 62% ($\pm$1.5; $n = 6$) after 6 months (Fig. 3b). The corresponding values for machined c.p. Ti implants after 3 and 6 months were 51% ($\pm$2.5; $n = 6$) and 58% ($\pm$5; $n = 5$), respectively (Fig. 3a and b). An average of 19% ($\pm$3.5; $n = 6$) bone contact was observed, after 3 months (Fig. 3c), for cast Ti which increased to 45% ($\pm$3.5; $n = 6$) after 6 months (Fig. 3d). The respective values for machined c.p. Ti were 25% ($\pm$7; $n = 6$) (Fig. 3c) at 3 and 37% ($\pm$7; $n = 5$) (Fig. 3d) at 6 months. There were no statistically significant differences for the bone area and bone-implant contact parameters between cast and machined c.p. Ti implants after 3 and 6 months.

4. Discussion

The casting procedure is influenced by several factors such as the investment material, mold temperature and atmosphere in the casting equipment. Molten titanium reacts with the mold material resulting in the formation of surface reaction layers which contain P, Mg, Si, Al and O [8, 9]. The solubility of these elements in molten Ti is different from each other. Therefore, these elements are located in different depths of the cast surface, resulting in the formation of a layered structure [8, 9]. The casting procedure also results in a material with altered mechanical properties, including an increased surface hardness [6, 8]. Provided that the surface properties of cast Ti do not convey an inferior biological response, some of the mechanical properties may prove advantageous in certain dental and medical applications. In the present study, the analysis of the surface of cast Ti and machined c.p. Ti showed a similar surface TiO_2 composition, covered with small amounts of contaminants, mainly hydrocarbons. Further, the estimates of the oxide thickness (2–5 nm) were similar for the two preparations and in agreement with previous data from machined titanium samples [16–20]. The present AES results of cast Ti are in good agreement with previous studies of differently prepared surfaces, made from cp Ti rod material [21]. Compared to the samples used in our previous studies [18, 19, 21] the present samples showed intermediate overall carbon levels and comparable levels of inorganic impurities. The AES results showed that the different surface preparations had not produced any major differences in chemical composition nor in oxide thickness between the samples.

In a previous study on the healing around cast Ti implants in soft tissue (abdominal wall of rats) a greater number of multinuclear giant cells was found around plugs made of non-machined cast Ti than c.p. Ti, possibly due to the differences in the surface chemical composition and/or topography [11]. In the present study the machining of cast Ti resulted, not only in a similar surface composition to that of c.p. Ti, but also in a similar bone response. The average percentage of bone area and bone-implant contact for machined cast Ti was similar to that of machined c.p. Ti after both 3 and 6 months. Therefore, as can be observed from the relative element concentrations in Table I, the machining process seemed to have eliminated a great portion of the surface contaminants observed in cast Ti [8]. However, since the casting procedure and the extension of the incorporated elements from the mold material into the

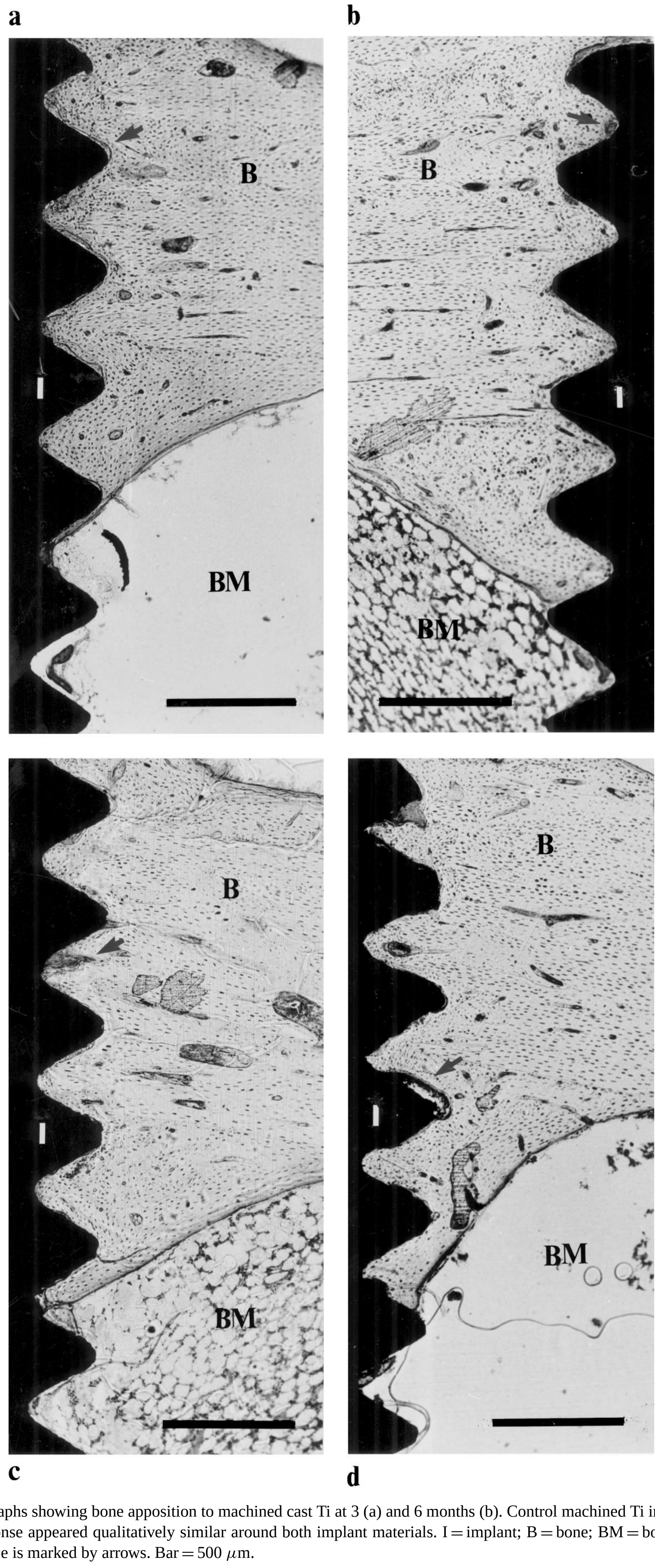

Figure 2 Light micrographs showing bone apposition to machined cast Ti at 3 (a) and 6 months (b). Control machined Ti implants at 3 (c) and 6 (d) months. The bone response appeared qualitatively similar around both implant materials. I = implant; B = bone; BM = bone marrow. Some of the soft tissue at the interface is marked by arrows. Bar = 500 μm.

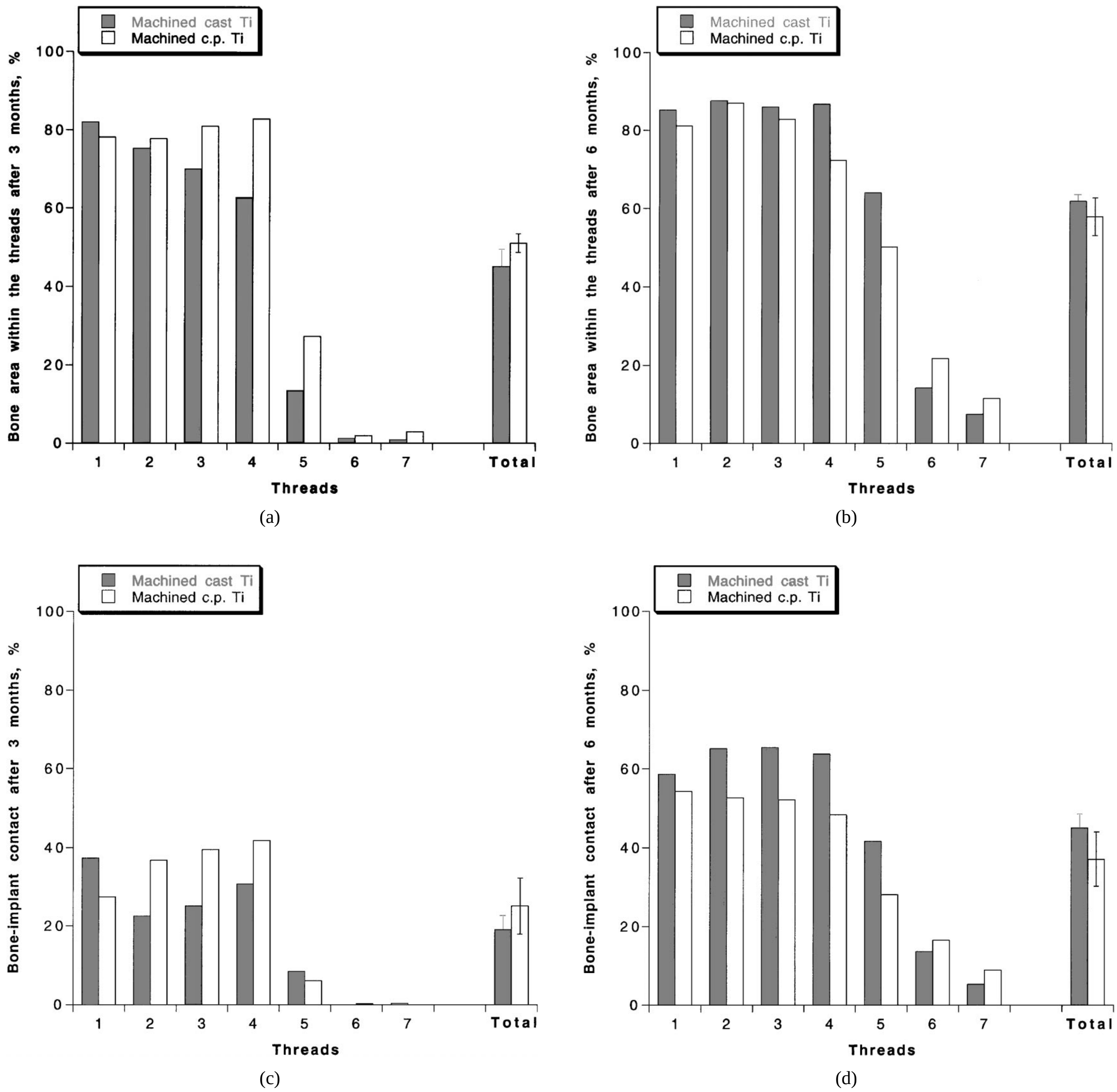

Figure 3 Light microscopic morphometry of ground sections. The percentage of bone area and bone contact in each of the threads (1–7) and for the entire implant section (total). (a) Bone area after 3 months. (b) Bone area after 6 months. (c) Bone contact after 3 months. (d) Bone contact after 6 months. No statistical difference in bone response toward the implanted materials could be observed.

molten Ti are not predictable, it is not possible to draw a definite conclusion as to how comprehensive the machining process should be in order to ensure a surface composition free of abundant contaminants. Therefore, it might be prudent to suggest that the composition of the mold material used for casting Ti as implant material should be based on materials which are known to be biocompatible such as Zr [22].

In addition, it is known that that microhardness of cast Ti is progressively reduced from the surface to the inner portion of the metal [2, 7, 8]. The increased microhardness of the superfical layers of cast Ti is believed to be partly due to the "alloying" of Ti with some of the contaminants derived from the mold [2, 7]. Thus, it can be assumed that while the machining procedure has removed a substantial amount of contaminants, present in the external layers of cast Ti, it may have partly decreased the advantageous mechanical properties of cast Ti. Further investigations are needed to determine the ideal mold and investment materials, the predictability of the post-casting treatment procedures, the reliability of machining procedures to eliminate contaminants from the superficial layers of cast Ti and the mechanical properties of machined cast Ti.

5. Summary

Machined, cast and conventionally machined Ti showed similar surface composition and oxide thickness. After 3 and 6 months implantation periods in rabbit cortical bone, histomorphometry did not reveal any significant differences in bone-implant contact and amount of bone filling the threads. These experimental observations indicate that machined, cast Ti implants have equally interesting beneficial properties as a bone implant material as clinically used Ti.

Acknowledgements

The authors would like to thank Annkristin Blomgren, Gunnel Bokhede, Lena Emanuelsson, Bernt Johansson and Badrudin Bhanji for their skilful technical assistance. We also wish to acknowledge Anders Oden for help with the statistical evaluation and Professor Karl-Gustav Strid for scientific advice. This study was supported by the Inga-Britt and Arne Lundberg's Science Foundation, the King Gustav V 80-year Fund, the Swedish National Association Against Rheumatism, the Faculties of Medicine and Odontology, University of Göteborg, The Swedish National Board for Technical Development (NUTEK), The Swedish Medical Research Council (9495), the C. M. Lerici Foundation, the Göteborg Medical Society and the Hjalmar Svensson Foundation.

References

1. H. HAMANAKA, H. DOI, T. YONEYAMA and O. OKUNO, *J. Dent. Res.* **68** (1989) 1529.
2. M. TAIRA, J. B. MOSER and E. H. GREENER, *Dent. Mater.* **5** (1989) 45.
3. M. ESPOSITO, in "Titanium in Medicine" (Springer Verlag, Heidelberg, 2001).
4. P. L. BLOCK and M. G. WILEY, *J. Prosthet. Dent.* **60** (1988) 221.
5. D. M. BELLES and P. L. BLOCK, *ibid.* **70** (1993) 1.
6. H. KIMURA, C. J. HORNG, M. OKAZAKI and J. TAKAHASHI, *Dent. Mater. J.* **9** (1990) 91.
7. J. TAKAHASHI, H. KIMURA, E. P. LAUTENSCHLAGER, J. H. CHERN LIN, J. B. MOSER and E. H. GREENER, *J. Dent. Res.* **69** (1990) 1800.
8. L. WICTORIN, N. EL-MAHALLAWY, M. A. TAHA and H. FREDRIKSSON, *Cast Metals* **4** (1992) 182.
9. O. MIYAKAWA, K. WATANABE, S. OKAWA, S. NAKANO, M. KOBAYASHI and N. SHIOKAWA, *Dent. Mater. J.* **8** (1989) 175.
10. R. BLACKMAN, N. BARGHI and C. TRAN, *J. Prosthet. Dent.* **65** (1991) 309.
11. S. MOHAMMADI, L. WICTORIN, L. E. ERICSON and P. THOMSEN, *J. Mater. Sci. Mater. Med.* **6** (1995) 435.
12. L. E. DAVIS, N. C. MACDONALD, P. W. PALMBERG, G. E. RIACH and R. E. WEBER, "Handbook of Auger Electron Spectroscopy," Eden Prairie: Physical Electronics Industries, Inc, 1976.
13. K. DONATH and G. BREUNER, *J. Oral Pathol.* **11** (1982) 318.
14. G. D. DAVIS, M. NATAN and K. A. ANDERSON, *Appl. Surf. Sci.* **15** (1983) 321.
15. J. LAUSMAA, *J. Electr. Spectr. Rel. Phen.* **81** (1996) 343.
16. J. LAUSMAA, B. KASEMO, U. ROLANDER, L. M. BJURSTEN, L. E. ERICSON, L. ROSANDER and P. THOMSEN, in "Surface Characterization of Biomaterials" (Elsevier Science Publishers B. V., Amsterdam, 1988) p. 161.
17. J. LAUSMAA, B. KASEMO and H. MATTSSON, *Appl. Surf. Sci.* **44** (1990) 133.
18. C. LARSSON, P. THOMSEN, B. O. ARONSSON, M. RODAHL, J. LAUSMAA, B. KASEMO and L. E. ERICSON, *Biomaterials* **17** (1996) 605.
19. C. LARSSON, L. EMANUELSSON, P. THOMSEN, L. E. ERICSON, B.-O. ARONSSON, B. KASEMO and J. LAUSMAA, *J. Mater. Sci. Mater. Med.* **8** (1997) 721.
20. M. ESPOSITO, J. LAUSMAA, J.-M. HIRSCH and P. THOMSEN, *J. Biomed. Mater. Res. Appl. Biomater.* **48** (1999) 559.
21. C. LARSSON, P. THOMSEN, J. LAUSMAA, M. RODAHL, B. KASEMO and L. E. ERICSON, *Biomaterials* **15** (1994) 1062.
22. P. THOMSEN, C. LARSSON, L. E. ERICSON, L. SENNERBY, J. LAUSMAA and B. KASEMO, *J. Mater. Sci. Mater. Med.* **8** (1997) 653.

Received 31 January
and accepted 4 October 2000