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Comparison of the tissue reaction to implants made of a beta titanium alloy and pure titanium. Experimental study on rabbits

A. UNGERSBÖCK, S. M. PERREN
AO/ASIF Research Institute Davos, CH-7270 Davos Platz, Switzerland
O. POHLER
Stratec Medical, CH-4437 Waldenburg, Switzerland

Commercially pure titanium (Ti cp) has been used successfully as an implant material in fracture fixation devices for many years. Ti cp is comparatively soft, but the mechanical properties, such as strength and ductility, can be adjusted by different means over a wide range. Titanium changes its crystal structure from a hexagonal (alpha) phase to the cubic (beta) phase at about 882 °C. Cubic titanium has the advantage of being very malleable (ductile), but in order to stabilize it at room temperature, additions of suitable alloying elements are required. In this study the soft tissue reaction to implants made from a beta titanium alloy (Ti–Mo–Zr–Al) with four different surface treatments is evaluated. The results are compared to Ti cp implants having the same surface conditions, and to electropolished stainless steel plates as controls. A minimum of four small plates of each group were implanted in rabbit tibiae for 3 months. Histomorphometric results show that the thickness of the soft tissue reaction layer, and the number of blood vessels, connective tissue cells (fibroblasts, fibrocytes), lymphocytes, and foreign body giant cells are not significantly different between beta titanium and Ti cp plates. For stainless steel plates the soft tissue reaction layer is thicker, and the numbers of macrophages and connective tissue cells are higher. Excellent biocompatibility was observed for this beta titanium alloy. The mechanical properties of this alloy surpass those of Ti cp, and because of the good tissue tolerance, this material seems to be advantageous and should enter into clinical testing.

1. Introduction

Commercially pure titanium (Ti cp) has been used successfully as an implant material in fracture fixation devices for many years [1–3]. Although the mechanical properties of Ti cp, such as maximum strength and ductility, are somewhat lower than those of work-strengthened stainless steel, titanium has attractive properties as an implant material. It has high corrosion resistance due to a spontaneously forming protective oxide surface layer [4–6], excellent biocompatibility [2, 7–10], low elastic modulus, and low weight. The mechanical properties of Ti cp can be varied in a wide range by the content of trace elements (interstitials) and mechanical deformation. Alloying of the titanium with other elements is another means to improve its strength. If good ductility and biocompatibility are requested at the same time, the choice of elements has to be very selective. Titanium changes its crystal structure at a certain temperature, a property also shown by iron and responsible for its versatility. The common hexagonal alpha structure of Ti transforms into a cubic beta structure at about 882 °C. The cubic beta titanium offers high malleability (ductility) but can only be stabilized at room temperature by the addition of other elements. An alloy composition of Ti–15Mo–5Zr–3Al (TAMZ) [4] has a stabilized beta phase and consists of elements known for their tissue tolerance. Metallurgical studies have shown that the material had variable mechanical properties with good workability suitable for surgical implants. Vanadium is excluded from this alloy because of its high cytotoxicity threshold when tested as an individual metal [11].

Before internal fixation devices made of new materials are implanted in the human body, the biocompatibility of such materials has to be demonstrated. Prior to *in vivo* testing the characterization of the surface roughness is recommended, since this has an important influence on the soft tissue reaction at the interface. In a long-term study in dogs the reaction of bone to screw implants made of Ti–Mo–Zr–Al (TAMZ) has been tested and was found to be favourable [12]. The present study is concerned with the soft tissue reaction to this beta titanium alloy.

Quantitative histological assessment of tissue reaction layers to implant surfaces has shown that the thickness of the reaction zone and the cell population are sensitive measures for evaluation of the tissue

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response [13–15]. Because relative tissue motion at the implant tissue interface and the implant surface preparation influence the results, in this study four different implant surface conditions, as relevant to actual implants, have been investigated. The surface structure of the implants are characterized by surface roughness measurements (profilometer) and scanning electron microscopy (SEM). Similar studies have been carried out previously on a larger series of titanium cp implants in rabbits using the same test protocol [16]. Therefore, histologic comparison is possible with larger number of test results than those included in the present study.

2. Materials and methods

2.1 Test material

For evaluation of the soft tissue response at the implant interface the following three-hole bone plates of dimensions $35 \times 5 \times 1$ mm were prepared for application to rabbits.

- Beta titanium alloy Ti–15Mo–5Zr–3Al (TAMZ) in a condition suitable for bone screws and plates.
- Titanium cp (commercial pure) of implant quality as used for AO-plates and screws corresponding to ISO 5832-2.
- Implant stainless steel 1.4441 (SS) corresponding to ISO 5832-1 in the work-strengthened condition as used for AO-bone plates and screws.

The TAMZ and titanium cp plates were prepared to give the following surface conditions:

(a) etched – plates were tumbled and etched to give a semirough surface;
(b) anodized – plates were tumbled and anodized to give roughness similar to that used for clinical osteosynthesis implants (as commercially applied by Stratec Medical);
(c) handground – plates were ground by hand, without further mechanical surface treatment, to give a uniform semirough structure without pores;
(d) electropolished – a smooth surface with rounded-off mechanical traces.

The stainless steel plates were electropolished in accordance with standard implant conditions to give a mirror-like surface.

The TAMZ and Ti cp plates were mounted with standard 1.5 mm cortical bone screws of Ti cp as used in clinical applications. The SS plates were mounted with standard 1.5 mm SS cortical bone screws as used in clinics.

2.2. Test methods

36 plates were tested in 18 adult rabbits. There was no particular preparation for the experimental animals other than the routine guidelines prior to an operation (no food for 12 h before the operation, water *ad libidum*). Anaesthesia was carried out in intubation (Halothan). Both hind legs of each rabbit were prepared for the operation by shaving and disinfection. The operation was carried out under sterile conditions with the same precautions as in clinics. The plates were mounted at the anteromedial site of the tibia under an extensor muscle, using two 1.5 mm monocortical screws. An equal distribution of the types of plates was made between right and left legs, and in each rabbit two different types of plates were implanted with selection of material based on statistical principles. Four plates of each type were tested. No administering of antibiotics prior to the operation nor during the test period took place.

After 3 months the rabbits were sacrificed using an overdose of narcotics. Careful preservation of the soft tissue covering the plate was observed. The implants were harvested with an intact tissue envelope and were left *in situ* for histological preparation.

2.3. Histological procedures

The bone/implant/soft tissue block was fixed in formalin 10% buffered with Na_2PO_4, dehydrated with alcohol and xylene and embedded in methylmethacrylate. The blocks were sectioned perpendicular to the longitudinal axis of the plate cutting through the centre of the plate. Sections of 6 μm thickness were cut with a Zeiss microtome, Giemsa stained, and mounted on glass slides for evaluation under the optical microscope.

The following parameters were determined. Measurements of the thickness of the soft tissue reaction layer adjacent to the implant. Counting of different cell types according to their morphological structure and staining behaviour [17]: connective tissue cells (CTC; fibroblasts, fibrocytes); macrophages (MP); round cells (RC; lymphocytes, plasma cells); mast cells (MSC); and foreign body giant cells (FBGC). Often it was difficult to separate the individual FBGC, therefore it was decided to count the nuclei of this cell type. Based on the staining used, only MP containing phagocytosed material were recognizable. Cells which circulate in blood (RC) were only counted when localized in the tissue or in the vessel wall; they were not counted when localized intravasally. For statistical reasons the histological sections were analysed "blind" without knowledge of the type of implant. The population of vessels and different cell types were presented in number/mm^2 and the FBGC in number/mm contact length with the implant surface. More details about the evaluation method are described elsewhere [18].

2.4. Statistical procedures

For the calculation of the number of microscopic fields to be evaluated in each sample, statistical methods were applied [19]. On average 21 microscopic fields per sample were evaluated. For evaluation of the significance of the results a double-sided standard *t*-test and a Newman–Keuls multiple range test (variance analysis) were applied.

2.5. Surface characterization

To characterize the surface roughness, profilometer measurements and scanning electron microscopy were

employed. Details of these techniques for characterization of the surface were recently described [20]. The roughness measurements were done on a profilometer of the Taylor–Hobson type with a four-sided pyramidic tip of 4 μm diameter. Three plates of each type as originally packed were randomly chosen for the measurements. Five measurements each per sample were carried out transversely to the long axis of the plates on the surface intended to be in contact with the soft tissue [21]. The following parameters were measured:

Rt – ISO 4287/1, Din 4761/1 (maximum roughness height between a peak and a valley)
Rtm – ISO 4287/1, Din 4761/1 (average of roughness height between a peak and a valley)
Ra – ISO 4287/1, Din 4761/1 (arithmetic mean of the roughness height)
Sm – ISO 4287/1, Din 4761/1 (arithmetic mean of the groove distance)

3. Results

3.1. Implant surface topography

The results of the roughness measurements for each type of plate are summarized in Table I: each value represents the mean of 15 individual measurement profiles per type of plate. These results for the five measurements on each plate and among the three plates of the same type show uniform and constant results, indicated by a low standard deviation. In general, the roughness parameters for the etched, anodized, and handground TAMZ and Ti cp implants are in the same range. The electropolished SS plates have the smoothest surface. As for clinical SS implants the electrochemical polishing process is well defined and reproducible. Together with the structural cleanliness of the implant steel, perfectly polished surfaces can be achieved. For the TAMZ and Ti cp, electropolishing is a rather special process and was conducted on an experimental basis, therefore, the smoothness is not of the same high quality as for the SS plates. Fig. 1 shows the electropolished TAMZ surface, indicating

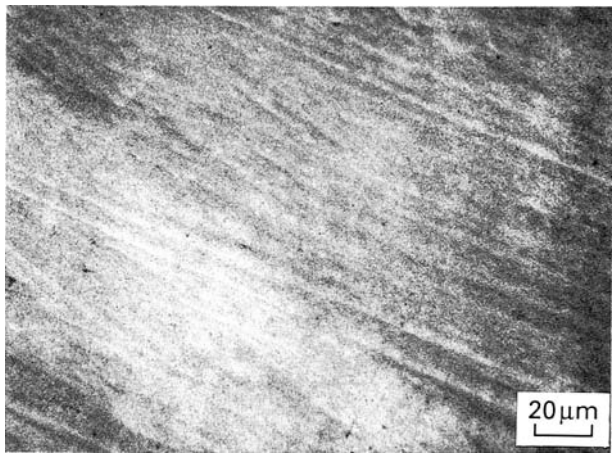

Figure 1 SEM micrograph of electropolished Ti–Mo–Zr–Al alloy. Smooth surface with mechanical traces rounded off by the electropolishing process.

TABLE I The results (mean value ± standard deviation) of the roughness parameters for each type of plate. *Rt* = maximum roughness height between a peak and a valley, *Rtm* = average of roughness height between a peak and a valley, *Ra* = arithmetic mean of the roughness height, *Sm* = arithmetic mean of the groove distance, Ti cp = titanium commerical pure, TAMZ = beta titanium alloy Ti–15Mo–5Zr–3Al.

Implant	*Rt*	*Rtm*	*Ra*	*Sm*
TAMZ etched	2.2 ± 0.9	1.4 ± 0.6	0.31 ± 0.1	86.9 ± 20.8
Ti etched	1.9 ± 0.2	1.5 ± 0.1	0.23 ± 0.02	23.8 ± 3.0
TAMZ anodized	2.2 ± 1.1	1.4 ± 0.5	0.29 ± 0.08	10.1 ± 25
Ti anodized	2.3 ± 0.3	1.7 ± 0.2	0.33 ± 0.06	43 ± 8.2
TAMZ handground	2.0 ± 0.9	1.2 ± 0.4	0.15 ± 0.05	16.8 ± 4.7
Ti handground	2.3 ± 0.5	1.7 ± 0.2	0.23 ± 0.01	19 ± 1.3
TAMZ electropolished	2.2 ± 1.02	1.2 ± 0.4	0.23 ± 0.06	91 ± 28
Ti electropolished	1.3 ± 0.2	0.9 ± 0.1	0.19 ± 0.02	52 ± 6.7
SS electropolished	1.0 ± 0.6	0.5 ± 0.1	0.08 ± 0.02	48 ± 11

TABLE II The results (mean value ± standard error) of the histological parameters for all implants (capsule = thickness of the connective tissue reaction layer, BV = blood vessels, CTC = connective tissue cells (fibroblasts and fibrocytes), MP = macrophages, RC = round cells (lymphocytes, plasma cells), FBGC = foreign body giant cells).

Implant	Capsule (μm ± SEM)	BV/mm² ± SEM	CTC/mm² ± SEM	MP/mm² ± SEM	RC/mm² ± SEM	FBGC/mm ± SEM
TAMZ etched	52 ± 13	15 ± 5	790 ± 233	10 ± 5	21 ± 11	6.6 ± 1.9
Ti cp etched	50 ± 0	14 ± 2	571 ± 17	6 ± 2	14 ± 2	8.3 ± 0.2
TAMZ anodized	71 ± 13	12 ± 3	884 ± 187	8 ± 1	34 ± 16	6.7 ± 0.9
Ti cp anodized	95 ± 5	31 ± 1	1051 ± 76	26 ± 6	30 ± 13	4.5 ± 2.5
TAMZ handground	100 ± 50	22 ± 6	1001 ± 452	9 ± 3	13 ± 6	8.1 ± 6.4
Ti cp handground	109 ± 30	14 ± 3	1006 ± 262	11 ± 1	33 ± 12	14 ± 5.5
TAMZ electropolished	51 ± 10	17 ± 7	840 ± 197	10 ± 4	27 ± 9	4.1 ± 1.9
Ti cp electropolished	100 ± 20	26 ± 8	834 ± 101	21 ± 11	25 ± 4	15 ± 3.4
SS electropolished	138 ± 28	28 ± 4	1488 ± 290	102 ± 25	28 ± 4	17 ± 7

that mechanical traces of the original surface treatment are smoothed down by the electropolishing process.

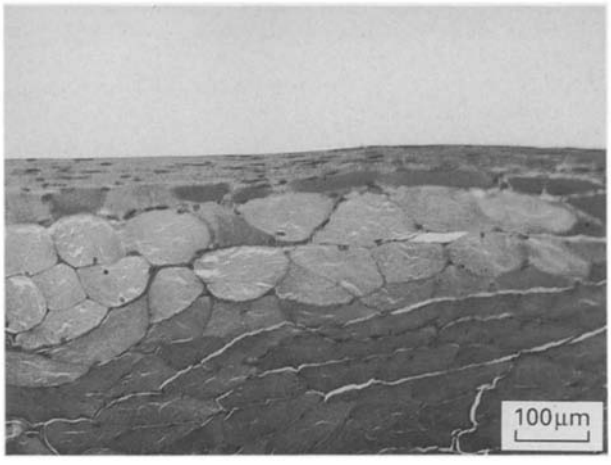

Figure 2 Optical micrograph of a histological section at the interface of the soft tissue to the implant surface of electropolished Ti–Mo–Zr–Al. A thin connective tissue reaction layer in contact with muscle tissue is observed.

3.2. Histologic evaluation

The electropolished steel plates show a continuous liquid film between the soft tissue and the plate; this was not observed for the electropolished TAMZ or Ti cp nor for the other surface treatments. Fig. 2 shows the tissue interface of the electropolished TAMZ, with a very small connective tissue reaction zone.

The results of the histomorphometric analyses are summarized in Table II. For the electropolished steel plates the soft tissue reaction layer covering the plate is thicker, the number of CTC is higher without being significantly different, and the number of macrophages is significantly higher compared to the other implants.

Between the different surface treatments of TAMZ and Ti cp implants, no significant difference is observed concerning the thickness of the soft tissue reaction layer. This is demonstrated in Fig. 3a and b, which compare the tissue interfaces of anodized beta alloy TAMZ and Ti cp, respectively. The tissue reactions are similar. The same is true for the handground TAMZ and Ti cp surfaces, Fig. 4a and b, respectively. The number of blood vessels, round cells, and foreign body giant cells are in the same range for all tested

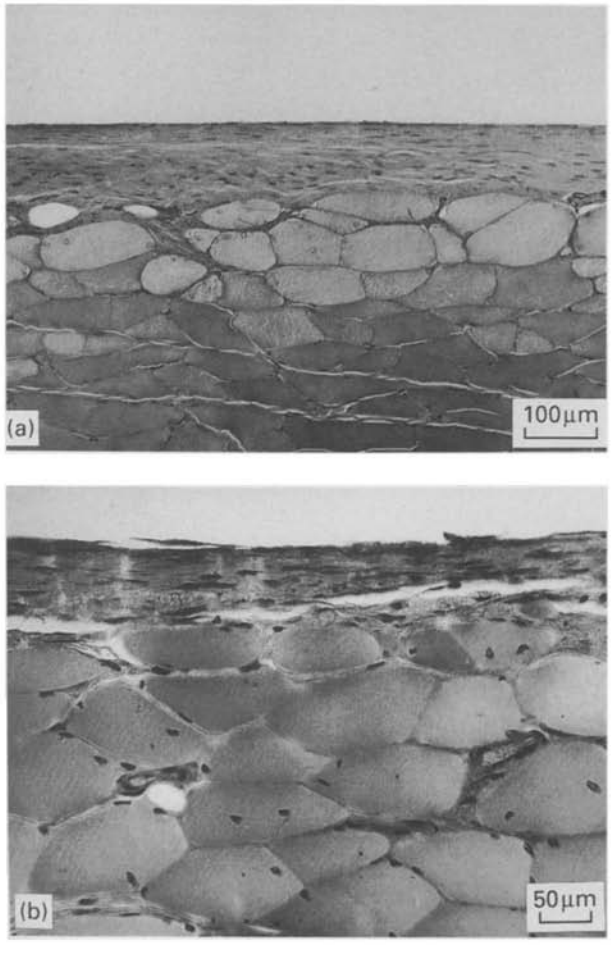

Figure 3 Optical micrographs of histological sections at the interface of the soft tissue to the implant surface of (a) anodized Ti–Mo–Zr–Al, and (b) anodized Ti cp. A thin connective tissue reaction layer in contact with muscle tissue is observed for both implant materials. The tissue reactions are similar for both materials.

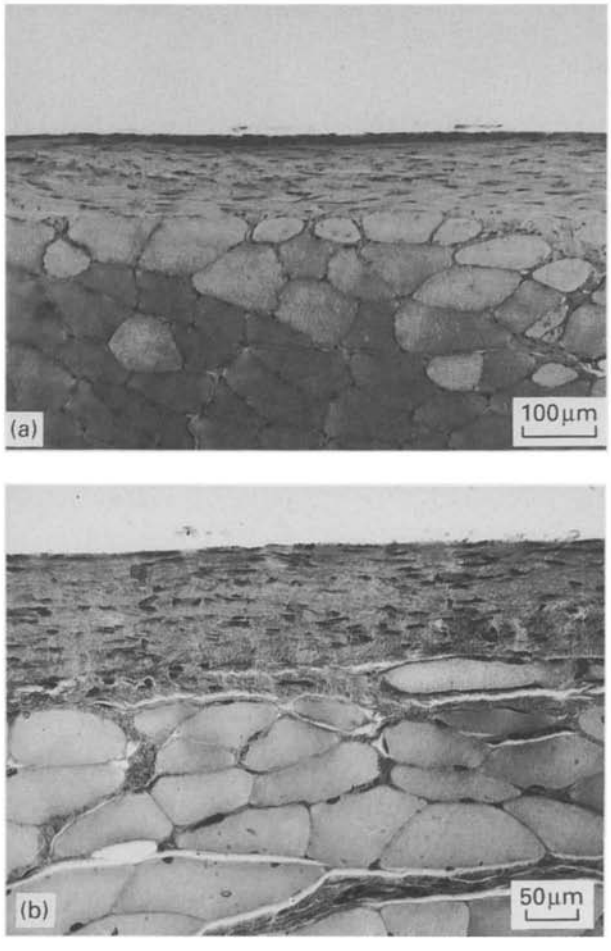

Figure 4 Optical micrographs of histological sections at the interface of the soft tissue to the implant surface of (a) handground Ti–Mo–Zr–Al, and (b) handground Ti cp. A thin connective tissue reaction layer in contact with muscle tissue is observed for both implant materials. The tissue reactions are similar for both materials.

implants. The macrophages are significantly higher for Ti cp than for TAMZ anodized plates.

4. Discussion

The implant material and the surface roughness determine the implant tissue interface and the tissue reaction. Both factors were considered in this study where the biocompatibility of beta titanium alloy (Ti–Mo–Zr–Al) was assessed in comparison to standard Ti cp material. The influence of the surface roughness was discussed earlier [16]. The soft tissue reaction to the TAMZ was as good as to the Ti cp, with no liquid film at the interface and with thin connective tissue reaction layers throughout. The same was found in an earlier study related to SS and Ti cp implant surfaces [16]. Although the soft tissue reaction to the electropolished SS plates still appears mild, a liquid film at the interface was observed and a comparatively thicker soft tissue reaction layer and increased numbers of CTC and MP were found.

The mechanical properties of the beta titanium alloy have advantages over the titanium cp for certain applications. Strength and fatigue resistance are increased, besides providing good ductility. Compared to alpha/beta Ti alloys like Ti–Al6–V4 the deformability during production and clinical application is improved. This study showed a tissue reaction for the beta titanium alloy which is as good as for pure titanium; the same was observed for studies on bone in dog [12]. Thus, this beta alloy appears to be an attractive implant candidate material and is ready to enter clinical investigation.

5. Conclusions

The beta titanium alloy (Ti–Mo–Zr–Al) showed excellent biocompatibility in the animal study. Since the mechanical properties of this implant material are favourable, clinical testing will follow.

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