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Quantitative bone tissue response to commercially pure titanium implants

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Commercially pure titanium implants were inserted in rabbit tibia for 3, 6 and 12 weeks. Each rabbit had two implants inserted, one for removal torque measurements and the other for histomorphometrical analysis. Light microscopic observations revealed that there was a continuing bone remodelling, with new bone formation in the periosteal region after 3 and 6 weeks, which diminished with time, i.e. up to 12 weeks of follow-up. A higher removal torque was observed with increasing time of implant insertion. The removal torque values for the 12 week samples were converted to three different shear forces depending on three different theoretically calculated implant to bone attachment levels. The mean shear forces related to the entire length of the implant surface was 0.6 $N\,mm^{-2}$. If considering the length of the implant inside the cortical bone only, the mean shear force was 1.9 $N\,mm^{-2}$, and if the bone–metal contact length as related to an estimate of the bone–implant contact a mean shear force of 14.8 $N\,mm^{-2}$ was calculated. Histomorphometrical measurements revealed more bone–metal contact as well as a larger bone area in the threads with increasing time of insertion. Statistically significant differences were observed between all measurements of the 3 and 6 week samples and between the 3 and 12 week samples. For the 6 and 12 week sections a statistically significant difference could be demonstrated only when comparing bone areas in the three best consecutive threads located in the cortical region.

1. Introduction

Commercially pure titanium is successfully used as an implant material in oral and orthopaedic reconstructive surgery. Excellent results of the bone tissue response to commercially pure titanium have been demonstrated in long-term oral and craniofacial studies [1–11]. Animal experiments based on histomorphometry have been performed that verify this favourable bone tissue response to commercially pure titanium in comparison with other metallic materials [12]. The aim of this study was to investigate further the interfacial reactions to commercially pure titanium by evaluating the stability of the bone bed with a removal torque unit and then to convert these measurements to shear forces. In addition, histomorphometrical data from similar implants inserted in the same animals were used to allow for evaluations of the importance of the nature of the interface.

2. Materials and methods

2.1. Animals and anaesthesia

Altogether 24 adult (average age 10 months) New Zealand White rabbits were used in this study. They were divided into three groups, with eight rabbits per group and were followed for 3, 6 or 12 weeks. For surgical procedures they were anaesthetized with intramuscular injections of fentanyl and fluanizon (Hypnorm Vet., Janssen Farmaceutica, Belgium) at a dose of 0.5 $ml\,kg^{-1}$ body weight and intraperitoneal injections of diazepam (Valium, Roche, France) at a dose of 2.5 mg $animal^{-1}$. Local anaesthesia with 1.0 ml 5% Xylocaine (Astra, Sweden) was administered to the proximal tibia, where the implants were to be installed under aseptic conditions. The shaved skin of the rabbits was washed with a mixture of iodine and 70% ethanol before surgery. At the preselected time of killing intravenous injections of a mixture of saline and barbiturates (Mebumal Vet., 60 $mg\,ml^{-1}$, Nord Vacc, Sweden) 1:4 were given.

2.2. Implants and surgical technique

A total of 48 screw-shaped implants with a square head and a total length of 10 mm were made from commercially pure titanium (99.75% purity) rods. They were manually manufactured by machining (tolerance $\pm$ 0.05 mm) to an outer diameter of 3.7 mm, a

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core diameter of 3.2 mm and a pitch height of 0.6 mm. The surface of the implants was examined with a scanning electron microscope (SEM, Jeol JSM-T300, Tokyo, Japan). The implant design, with a square head, enabled them to fit into a specially constructed pin which could be connected to a removal torque unit. After ultrasonic cleaning in trichloroethylene and absolute ethanol the implants were sterilized in an autoclave. A very gentle surgical technique was used, involving cutting the fasciae in separate layers, and bone penetration at low rotary drill speeds using sequential drill-bits and profuse cooling with saline. The insertion site was the proximal tibial methaphysis of the left leg. Two implants (inserted 5 mm apart) were allowed to penetrate the first cortical layer, never entering the opposite side and leaving two threads visible above the cortex. The fascia and skin were sutured in separate layers with Vicryl 5-0 and Ethicon 3-0 silk. No external bandages were applied. Immediately after surgery the rabbits were allowed full weight-bearing.

2.3. Biomechanical tests, preparation of specimens and histomorphometry

On the day on which the rabbits were killed they were again anaesthetized and the skin and fasciae were opened. In order to investigate the implant stability, a torque gauge (Tohnichi 6 BTG-N, Tohnichi MFG Co., Ltd, Tokyo, Japan) instrument was used, which enabled direct readings of the necessary removal torque in N cm. The distal implant was always chosen for biomechanical measurements, i.e. the implant was unscrewed, the torque registered and the implant was left in the bone bed. The removal torque technique involved a slow gradual increase of the applied torque until implant loosening occurred. The removal torque (in N cm) measurements from the 3 months group were also converted into shear forces (N mm^{-2}) according to the formula $T/\pi dlr_1$, where T is the removal torque in N mm, d is the mean diameter of the implant (3.45 mm), l the length (in the present paper we consider three different lengths, see Table II, below) and r_l is the lever arm (= radius 1.725 mm, see Fig. 9, below). Both the proximal and distal implants were then retrieved with surrounding bone for later histomorphometrical analyses. These specimens were fixed in 4% neutral buffered formaldehyde and further processed to be embedded in light-curing resin (Technovit 7200 VLC, Kulzer and Co., Germany). The cutting (of the long axis of the implant) and grinding (to a thickness of about 10 μm) was carried out in Exakt sawing and grinding equipment. The sections were stained using four parts 1% toluidine blue in a 1% borax solution mixed with one part 1% pyronin-G solution [13]. Light microscopic investigations were performed in a Leitz Aristoplan. The histomorphometrical calculations were performed with a computer-based system (Leitz Microvid equipment connected to a PC and a mouse). All measurements were performed "directly in the microscope", i.e. in the eyepiece of the microscope using a × 10 objective and a × 2.5 zoom when higher magnification was needed to clarify the picture. The histomorphometrical investigations involved calculations of the total bone to metal contact and the total bone area in the threads as well as for the three best consecutive threads (on each side of the sectioned implant) which represents the situation in the cortical passage. The "mirror-image" analysis was used to compare the bone area occupied "inside" a thread to a corresponding area immediately "outside" the same thread. Two consecutive threads in the mid-cortical region on each side of the sectioned implant were chosen for the mirror-image analysis. The mirror-image measurements are illustrated below in Fig. 10.

2.4. Statistics

For statistical calculations the Mann–Whitney U-test and Wilcoxon signed rank test were used.

3. Results

SEM revealed that the implant surfaces had micro-grooves, small pits and elevated areas (Fig. 1). A qualitative investigation of the histological 10 μm-thick ground sections was performed in the light microscope. Comparing the sections demonstrated a continuing remodelling of the bone tissue. The new bone formation in the periosteal region was obvious at 3 and 6 weeks and diminished with time, i.e. at 12 weeks of follow-up (Figs 2–4). The implants that had been inserted for 3 weeks were largely surrounded by soft tissue. The bone appeared immature in comparison with the 6 and 12 weeks samples (Figs 5–7). Irrespective of the implantation time, macrophages and multinucleated giant cells were observed close to the bone tissue and in close contact with the commercially pure titanium implants. Large elongated multinucleated giant cells were often observed in close contact with the implant surface facing the marrow cavity (Fig. 8).

After 3 weeks insertion the mean removal torque was 8.3 N cm (range 5–12.5 N cm). Six weeks insertion

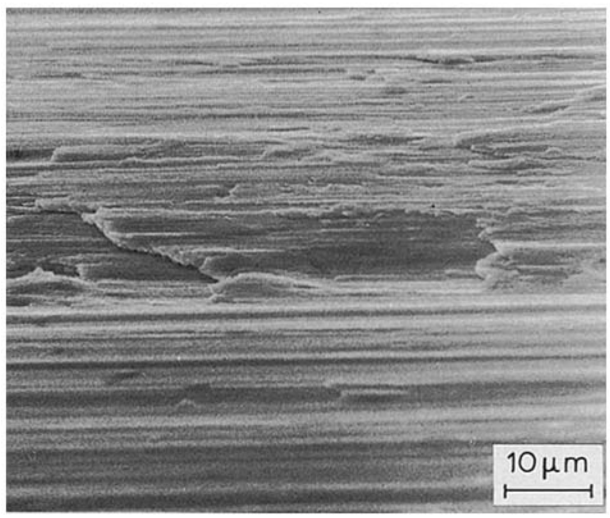

Figure 1 SEM micrograph of a commercial pure titanium implant. The longitudinal grooves and ridges are typical for machined surfaces.

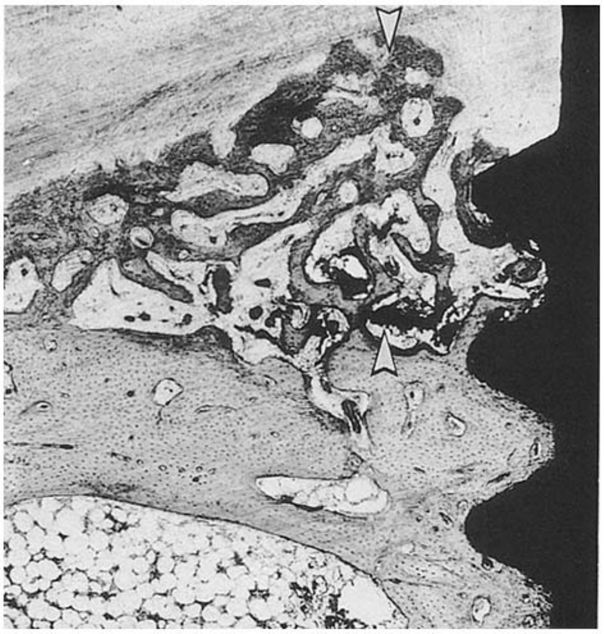

Figure 2 Survey light micrograph from a 3 week section. New bone formation (between arrow heads) in the periosteal region may be observed close to the top of the implant. There is a distance of 600 μm between the thread peaks.

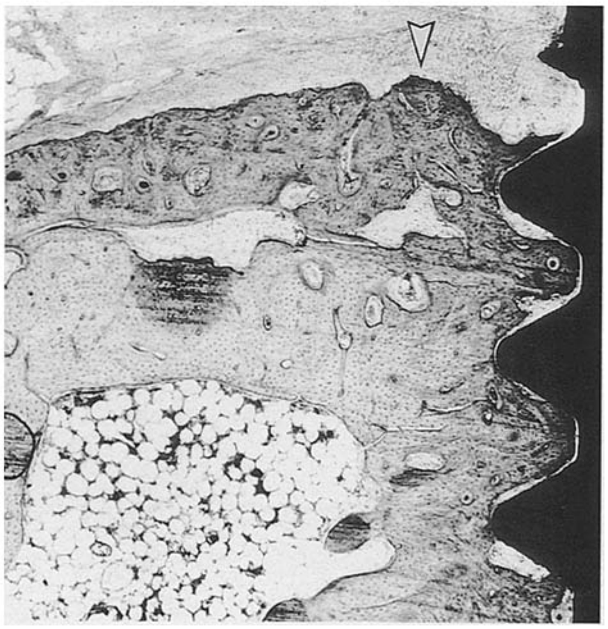

Figure 3 Survey light micrograph from a 6 week section. New bone formation (arrowhead) in the periosteal region may be observed. There is a distance of 600 μm between the thread peaks.

gave a mean removal torque of 15.8 N cm (range 9.5–21 N cm) and a mean of 19.9 N cm (range 15–24 N cm) was necessary to loosen the implants after an insertion time of 12 weeks. Statistical analysis (Mann–Whitney U-test) between the 3 and 6 week implants revealed a P-value of 0.003 and between the 3 and 12 week implants a P-value of 0.002, whereas comparisons between the 6 and 12 week implants showed a P-value of 0.1. The removal torque values are shown in Table I.

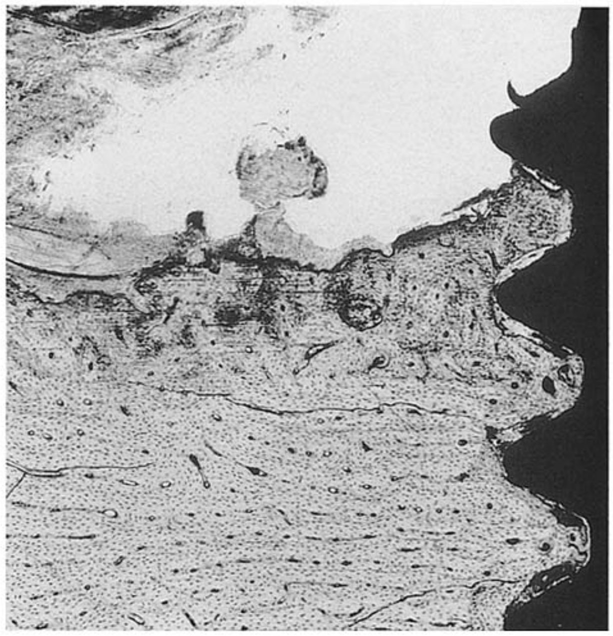

Figure 4 Survey light micrograph from a 12 week section. There is a distance of 600 μm between the thread peaks.

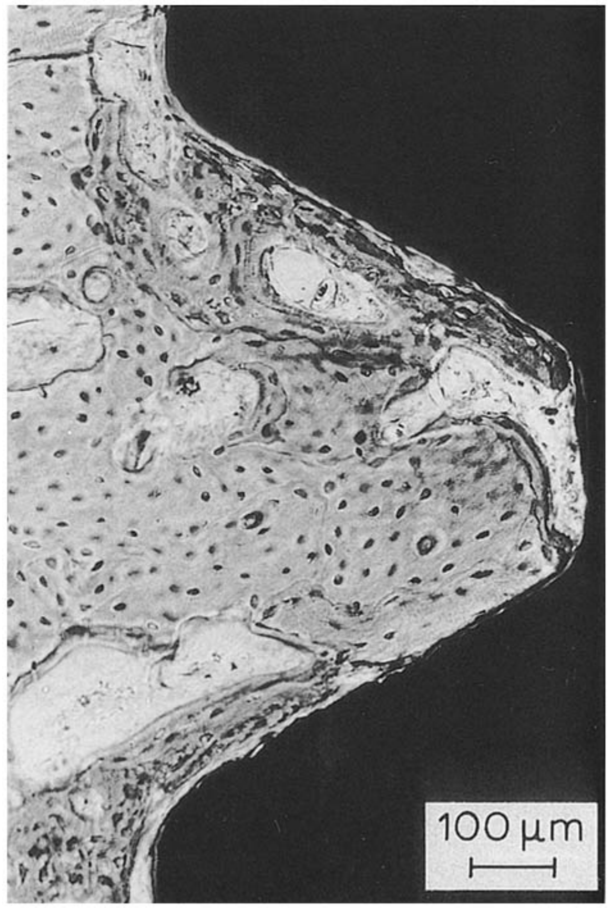

Figure 5 Light micrograph of a ground section from a 3 week sample. The bone appeared immature in comparison with the 6 and 12 week samples.

The removal torque values after 12 weeks were converted to shear forces. Three different lengths could be considered when converting to shear forces (Fig. 9): when the total length of the threaded part of

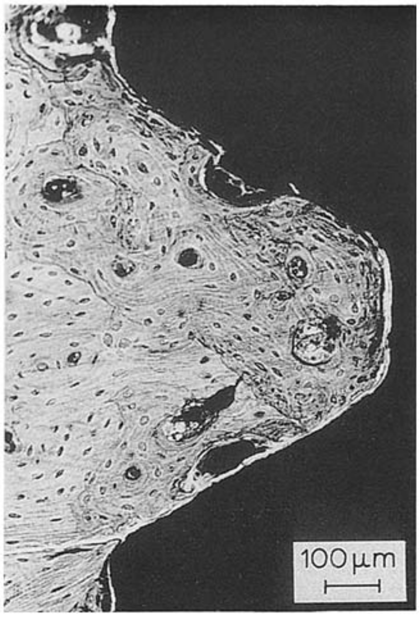

Figure 6 Light micrograph of a ground section from a 6 week sample. The bone tissue appears to be more woven than the 12 week samples.

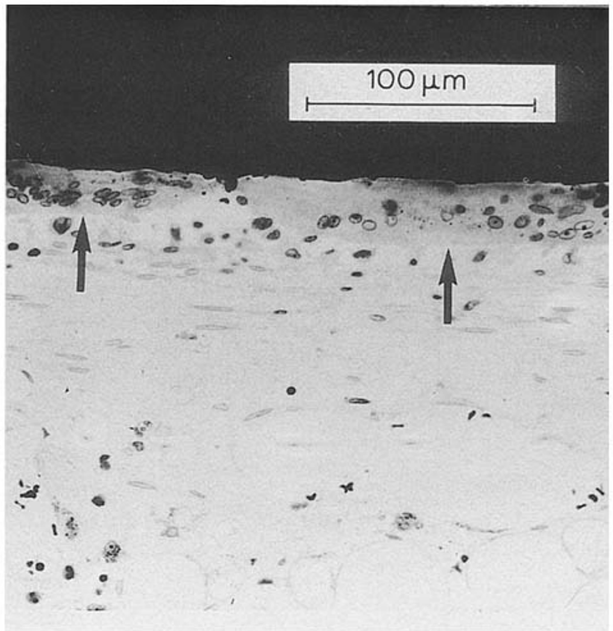

Figure 8 Multinucleated giant cells (arrows) appearing on the implant surface.

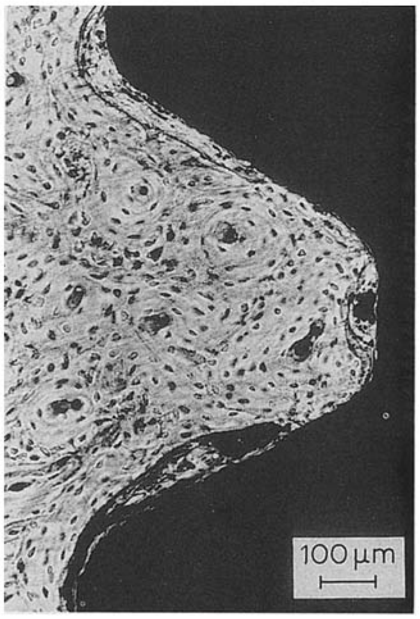

Figure 7 Light micrograph of a ground section from a 12 week sample. The bone tissue is mature in comparison with 3 and 6 week samples.

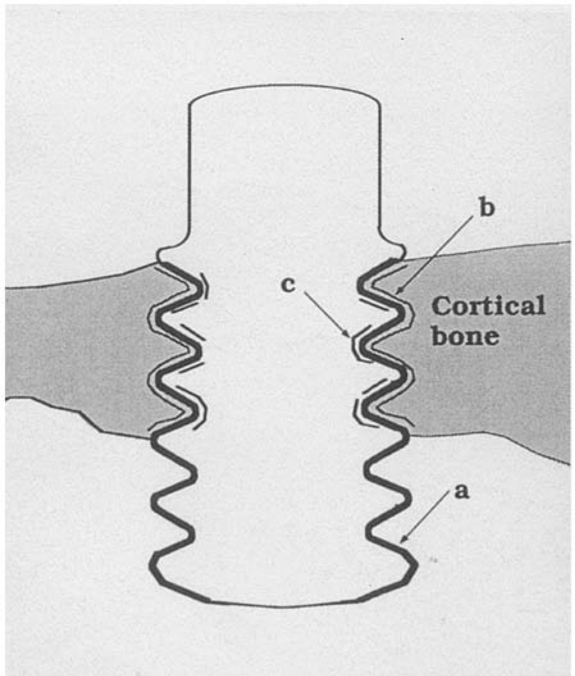

Figure 9 Schematic drawing of an implant inserted in the cortical layer of tibia, illustrating how the three different shear forces were considered: (a) the entire length of the threaded part of the implant, starting from the top at the periosteal level and ending in the marrow cavity; (b) the entire length of the implant surface in the cortical region; and (c) the bone–metal contact length measured on the not-removed "neighbour" implants.

the implant, i.e. starting from the top at the periosteal level and ending in the marrow cavity was used as a basis (a), there was a mean of 0.6 N mm^{-2} (range 0.4–0.8 N mm^{-2}); when, on the other hand, only the length of potential cortical contact layer was used for comparisons (b) (i.e. the total implant length in the cortical region) a mean shear value of 1.9 N mm^{-2} (range 1.4–2.2 N mm^{-2}) was found; shear forces, based on the calculations of the total bone–metal contacts (c) (on the not-unscrewed proximal implants) revealed a mean shear force of 14.8 N mm^{-2} (range 8.1–21.4 N mm^{-2}). The three different shear forces obtained are given in Table II.

Calculations of the bone–metal contact were performed along the entire surface, revealing a mean of 1.3% (0–5.9%) for the 3 week samples. For the 6 week sections the mean was 12.0% (7.0–20.4%), and for the 12 weeks sections a mean of 13.5% (8.7–18.9%) bone–metal contact was obtained. Statistical calculations of the bone–metal contact between the 3 and 6 week measurements revealed a statistically significant difference ($P = 0.0002$). This was also the case when comparing the 3 week with the 12 week sections ($P = 0.0002$). Comparisons between the 6 and 12 week measurements revealed no statistically significant differences ($P = 0.506$). A selection of the three best consecutive threads at the cortical passage demonstrated a mean bony contact of 1.8% (0–6.9%) after 3 weeks insertion. At 6 weeks implant insertion a mean of 16.1% (9.8–26.3%) was obtained in the three best consecutive threads, whereas after 12 weeks a mean of 21.8% (12.3–30.9%) was demonstrated in the three best consecutive threads. Comparing the 3 and 6 week measurements and the 3 and 12 week measurements revealed a statistically significant difference in both cases ($P = 0.0002$), whereas no statistically significant difference was observed between the 6 and 12 week samples ($P = 0.130$). Table III demonstrates the bone–metal contacts. Calculations of the total bone area in the threads demonstrated for the 3 week samples a mean of 42.5% (30.9–51.9%), for the 6 week sections a mean bone area of 61.1% (53.6–70.4%) was obtained and in the 12 week sections a mean of 57.7% (45.3–75.7%) was demonstrated. Statistically significant differences were obtained between the 3 and 6 week measurements ($P = 0.0002$) and between the 3 and 12 week measurements ($P = 0.0018$), whereas no statistically significant differences could be observed between the 6 and 12 week measurements ($P = 0.386$). Selecting the bone area in the three best consecutive threads on each side of the implant demonstrated, in the 3 week cases, a mean of 53.2% (41.2–66.0%). After 6 weeks insertion a mean of 73.15% (66.1–77.5%) was obtained and after 12 weeks a mean of 79.4% (70.8–87.9%) could be demonstrated. Statistically significant differences were obtained between the 3 and 6 week ($P = 0.0002$), the 3 and 12 week ($P = 0.0002$) and the 6 and 12 week ($P = 0.0046$) comparisons. Table IV shows the calculation of the bone areas. Table V shows the statistical results obtained when comparing bone–metal contacts and bone areas at different time intervals, i.e. 3 weeks with 6 weeks, 3 weeks with 12 weeks and 6 weeks with 12 weeks. The results from the mirror image analysis (Fig. 10) revealed that there were statistically significant differences between the amount of bone occupying these threads in all three groups. There was more bone in the outer areas compared with in the inner areas. After 3 weeks the mean percentage bone area "inside" was 70.0% and

TABLE I Removal torque (N cm) of commercial purity titanium implants after different times of insertion in rabbit tibia. Each group consisted of eight New Zealand White rabbits

Rabbit no.	Group 1 (3 weeks)	Group 2 (6 weeks)	Group 3 (12 weeks)
1	7.5	20	21.5
2	12.5	13	20.5
3	9.5	21	19
4	12	20.5	24
5	5	15	18.5
6	9	16	20
7	5	9.5	15
8	6	11.5	20.5
Mean	8.3	15.8	19.8
SD	3.0	4.4	2.8
SEM	1.0	1.5	1.1

TABLE II Shear forces ($N\ mm^{-2}$) calculated based on the formula $T/\pi dlr_1$, where T is the removal torque (N mm), d is the mean diameter of the implant (3.45 mm), r_1 is the lever arm (= radius 1.725 mm) and l = (a) the entire length of the threaded part of the implant, starting from the top at the periosteal level and ending in the marrow cavity, (b) the entire length of the implant surface in the cortical region and (c) the bone–metal contact length measured on the not-unscrewed implants

	a	b	c
	0.62	1.99	14.13
	0.57	2.09	11.10
	0.56	1.98	19.42
	0.75	2.20	16.91
	0.55	1.41	8.06
	0.61	1.86	21.37
	0.53	2.08	12.39
	0.44	1.36	14.86
Mean	0.6	1.9	14.8
SD	0.1	0.9	4.4

TABLE III The bone–metal contact (%), around the entire implant and in the three best consecutive threads (3 best) at various times of follow-up

Rabbit No.	Group 1 (3 weeks)		Group 2 (6 weeks)		Group 3 (12 weeks)	
	Entire	3 best	Entire	3 best	Entire	3 best
1	3.2	5.3	7.0	9.8	14.1	21.1
2	5.9	6.9	20.4	26.3	18.9	30.9
3	0	0	11.1	18.5	10.2	17.4
4	0.5	0.6	15.0	19.3	13.0	23.8
5	0	0	7.4	9.8	17.5	30.7
6	0.8	1.2	11.2	13.3	8.7	14.5
7	0.3	0.4	12.5	16.2	16.8	23.9
8	0	0	11.0	15.6	9.2	12.3
Mean	1.3	1.8	12.0	16.1	13.5	21.8
SD	2.2	2.7	4.3	5.4	3.9	6.9
SEM	0.8	1.0	1.5	1.9	1.4	2.4

TABLE IV The bone area (%), around the entire implant and in the three best consecutive threads (3 best) at various times of follow-up

Rabbit no	Group 1 (3 weeks)		Group 2 (6 weeks)		Group 3 (12 weeks)	
	Entire	3 best	Entire	3 best	Entire	3 best
1	51.9	66.0	58.4	67.4	52.0	78.1
2	43.9	51.3	57.3	66.1	67.0	87.9
3	41.5	55.3	53.6	77.5	58.0	78.8
4	44.6	53.8	72.6	76.4	45.3	83.1
5	30.9	41.2	58.7	74.0	47.2	80.9
6	46.7	56.4	70.4	73.6	46.8	77.9
7	36.1	46.5	56.9	73.4	69.1	70.8
8	44.4	54.9	60.7	76.7	75.7	77.5
Mean	42.5	53.2	61.1	73.2	57.1	79.4
SD	6.5	7.3	6.8	4.3	11.7	4.9
SEM	2.3	2.6	2.4	1.5	4.1	1.7

TABLE V P-values obtained from the statistical tests (Mann–Whitney U-test) performed on the bone–metal contact (bmc) and the bone area (ba) on the entire (total) implant and the three best consecutive threads in the cortical passage

	3 weeks–6 weeks	3 weeks–12 weeks	6 weeks–12 weeks
Total bmc	0.0002	0.0002	0.506
Three best bmc	0.0002	0.0002	0.130
Total ba	0.0002	0.0018	0.382
Three best ba	0.0002	0.0002	0.0046

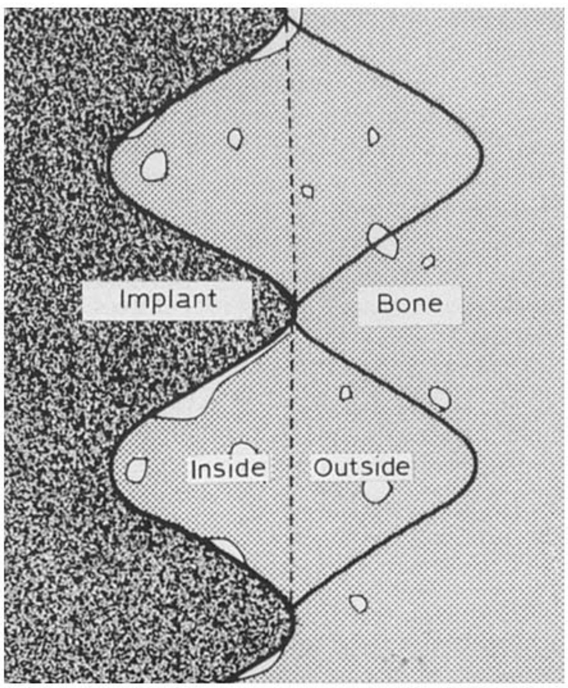

Figure 10 Schematic drawing of the "mirror-image", i.e. the bone area "inside" and "outside" a thread.

"outside" was 82.4% ($P = 0.02$). Six weeks after insertion the mean percentage bone area "inside" was 77.9% compared with "outside" 85.9% ($P = 0.02$). In the 12 week group means of 79.3% "inside" and 93.4% "outside" could be demonstrated ($P = 0.008$).

4. Discussion

This study demonstrated that there is more bone formed around commercially pure titanium implants with increasing time of follow-up. A previous study with similar types of implants as used in this study indicated a similar tendency of increasing removal torques with time [14]. Higher absolute values in the 1987 study compared with in the present study are probably explained by the use of different research animals, i.e. lop-eared rabbits with cortices twice as thick as the New Zealand White rabbits used in the present model. It must also be remembered that the torque gauge manometer is used in comparative studies (test to control) and that the lack of calibration makes comparisons between different raw values slightly uncertain. However, this methodological shortcoming is unlikely as an explanation for the considerable differences in raw values seen in the present study with removal torques in the range 5–20 N cm compared with the average of 35 N cm observed in another study with similar animals and a similar time of follow-up [15]. However, the latter study differed from the present one in that differently designed implants, a different surgical approach and a different removal torque technique were utilized. Sennerby *et al.* [15] were unable to detect any gradual increase in the removal torque with increasing time of follow-up, which in their experiment varied between 6 weeks and 6 months.

In the present experiment we converted our removal torque values to shear forces between the screw and the bone. This calculation was made with a simple formula based on the lever arm (= radius 1.725 mm) and the area of the screw in contact with the tissue (Table II). We calculated shear forces based on the total length of the threaded part of the implant, starting from the top at the periosteal level and ending in the marrow cavity. In this case a shear force of 0.6 N mm^{-2} (range 0.4–0.7 N mm^{-2}) was obtained.

However, this value may not be very realistic since in reality there was no bone at all in implant contact in the marrow space. If, on the other hand, we assume that all shear forces are located only at the cortical level, our mean shear force at 12 weeks was $1.9\,\mathrm{N\,mm^{-2}}$ (range 1.4–$2.2\,\mathrm{N\,mm^{-2}}$). However, in reality our histomorphometrical studies indicated a bone–implant contact of between 8.7 and 18.9% in our proximal (not unscrewed) implants of the same tibia, a finding that presumably also indicates the relative percentage of bone–implant contact in the distal removed implants, since measurements of the amount of bone inside the threads were quite similar in both cases (58.4% compared with 57.1%). Therefore, we calculated shear forces based on the bone–metal contact in the proximal insertion side; this would give a mean shear force of $14.8\,\mathrm{N\,mm^{-2}}$ (range 8.1–$21.4\,\mathrm{N\,mm^{-2}}$). These latter figures are those that we believe give the most realistic indication of the shear strength of the bone–titanium interface, given the design and surface of our used implants and reflecting only the situation of the bone at 3 months.

Steinemann *et al.* [16] measured the tear-off forces on rough and plasma-coated titanium implants in monkey bone. Depending on the surface of the implants, the tear-off force was in the range 1.0–$4.1\,\mathrm{N\,mm^{-2}}$. The same authors claim that the interface shear strength is about ten times higher, as deduced from the results of Claes *et al.* [17]. The latter authors measured the removal torque on smooth and rough ASIF AO-Cortical screws after being inserted in double cortical layers in sheep bone. Their smooth implants seemed to demonstrate a constant removal torque after 7, 28, 56 and up to 112 days, with a mean value of 32.4 N cm after 112 days insertion. During the same periods their rough implants demonstrated an increase in removal torque with time, being 44.1 N cm after 7 days and up to a mean value of 388 N cm after 112 days insertion. Our results seem to be in the same range as those described by Steinemann *et al.* [16] and Claes *et al.* [17], even if the latter authors demonstrated much higher removal torques than we observed. This may be explained by the different types of animals, different implant surfaces [18, 19] and the fact that the implants used by Claes *et al.* [17] penetrated both cortices of the implant site in contrast to the present study.

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