An animal study on the bone behaviour of Ca–P-coated implants: influence of implant location

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Four different implant materials were installed into the mandibular corner of goats to investigate the trabecular bone response in a mainly unloaded model. The implants were installed using a standardized technique and were left *in situ* for 12 weeks. One goat had to be sacrificed after surgery because of a broken rib; the other animals healed uneventfully. After sacrifice of the animals, the bone response to the uncoated and the three different Ca–P implants was evaluated histologically and histomorphometrically. Four sections of each implant were evaluated; two were located in the cortical and two in the trabecular bone. Of the 44 retrieved implants, 20 implants appeared to be installed partially in the mandibular canal, as clearly visible on the X-rays. These implants were not used in the histomorphometrical measurements. Histological evaluation showed that the trabecular and cortical bone reactions were similar; there was no significant difference in the percentage of bone contact nor in the amount of bone in contact with the implants. In conclusion this study showed that the mandibular corner is an unsatisfactory model for the installation of implants because of anatomical restrictions. Also, the experiment remained inconclusive about the influence of loading conditions on bone behaviour. Nevertheless, the histological results confirmed the bioactive properties of Ca–P coatings.

1. Introduction

Several clinical studies demonstrated that areas of jaws with a low density of bone, such as the posterior maxilla, offer significantly lower success rates compared with areas of denser structure [1–3]. Therefore, already several attempts have been made to improve implant anchorage in poor bone conditions. This has been done, for example, by the use of porous implant surfaces [4] or by the deposition of Ca–P coatings on the implant surface [5–10]. On the basis of these results it was concluded that Ca–P coatings, like hydroxyapatite and fluorapatite, result in a more rapid initial bone response and a greater bone adaptation compared with uncoated controls.

In addition to the biological aspect of Ca–P-coated implants, absence of implant mobility is a mechanical requirement for long-term fixation of implants [11]. This can be achieved by implant geometry that maximizes bone contact with the implant or by designs that allow bone ingrowth.

Considering the above-mentioned findings, it can be hypothesized that the newly formed bone on the implant surface is the result of biological and biomechanical processes that take place at the interface between bone tissue and the implant surface. In terms of bone remodelling activity this means that, besides implant surface properties, tensile or pressure forces will also influence bone formation [12]. In this context, Jensen [13] reported the hypertrophic bone response to stress imposed by titanium implants on the bone. Consequently, for the objective testing of the influence of implant surface properties on bone biocompatibility, research should be done in a mainly load-free situation to exclude the influence of stress and strain on the remodelling processes of bone tissue at the implant–bone interface [14, 15]. Although, for this purpose cell culture experiments can be done, extrapolation of *in-vitro* results to the *in-vivo* situation can be difficult. For example, Ca–P-coated implants not only are described as showing a favourable bone response but also are reported to be subject to substrate-to-coating fracture and coating degradation [16, 17]. Reliable information about these properties can only be obtained by *in-vivo* experiments [18].

Therefore, the aim of the present study was to compare four different implant materials installed in

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the trabecular bone of the mandibular corner of goats. This implantation region is described in the literature [19] as a neutral area, since load transmission is mainly confined to the thin layer of cortical bone whilst the contact area between the implant and the cancellous bone is virtually unloaded.

2. Materials and methods

2.1. Implant materials

48 cylindrical titanium alloy implants ($TiAl_6V_4$) were grit blasted with Al_2O_3 ($R_a = 4$–$5\ \mu m$). After grit blasting, 36 of the implants were coated with a Ca–P plasma-sprayed coating [5, 20], approximately 55–60 μm thick. The following coatings were deposited (Fig. 1): hydroxyapatite (HA); hydroxyapatite which was subsequently subjected to a heat treatment (650 °C for 10 min) (HAHT); fluorapatite (FA); uncoated titanium implants served as the control. The final diameter of all implants was 4 mm, and the length was 5 mm. After plasma spraying, the implants were cleaned ultrasonically in 100% ethanol. Finally, all implants were sterilized in an autoclave.

2.2. Experimental animal design and surgical technique

12 female Saane goats with an average mass of between 50 and 80 kg and an average age of 30 months were used. The animals were kept in quarantine for at least 4 weeks and tested for caprine arthritis encephalitis/CL arthritis. In each goat, all different implant types were installed in the mandible; two implants were inserted in the left and two in the right corner. The 48 implants were placed according to a balanced split-plot design: 12 HA coated, 12 HAHT coated, 12 FA coated and 12 Ti.

Surgery was performed under general anaesthesia induced by intravenous penthobarbital (25 mg kg^{-1}) and atropine (0.5 mg per animal). After orotracheal intubation, anaesthesia was maintained by ethrane (2–3%) through a constant-volume ventilator.

The animals were immobilized on their back for the insertion of the implants and the mandibles were shaved, washed and disinfected with povidone–iodine. The mandible corner was palpated and a longitudinal incision was made parallel to the direction of the masseter muscle. The bone was exposed by blunt dissection of the masseter muscle. Using a drill guide, two pilot holes were drilled at a fixed distance of 2 cm starting from the mandibular corner on the line connecting that corner to the lateral eye corner, and at 0.5 cm perpendicular to that line. The holes were gradually widened with drills to the final diameter of the implants to ensure firm fixation of the implants. The bone preparation was performed using a very gentle surgical technique and continuous internal cooling. Following press-fit insertion of the implants, the soft tissues were closed in separate layers using resorbable sutures (Vicryl 2-0). To reduce the perioperative infection risk, the prophylactic antibiotic Albipen® was administered for 3 days starting 1 h post-operatively.

2.3. Histological procedures

The animals were sacrificed 12 weeks after implant installation using an overdose of Nembutal® and the mandibles with the implants were retrieved. Excess soft and hard tissue was removed immediately to reduce the samples to smaller specimens and these samples were fixated in 10% buffered formalin solution. To confirm the position of the implants in the mandibular corner, long-cone radiographs were taken of the samples, perpendicular to the long axis of the implants (Fig. 2). The specimens were dehydrated by

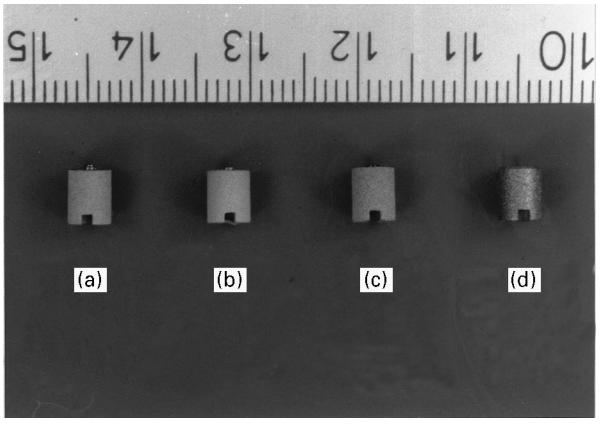


Figure 1 Photograph of the four different implant types: (a)–(c) Ca–P-coated implants; (d) uncoated titanium implant.

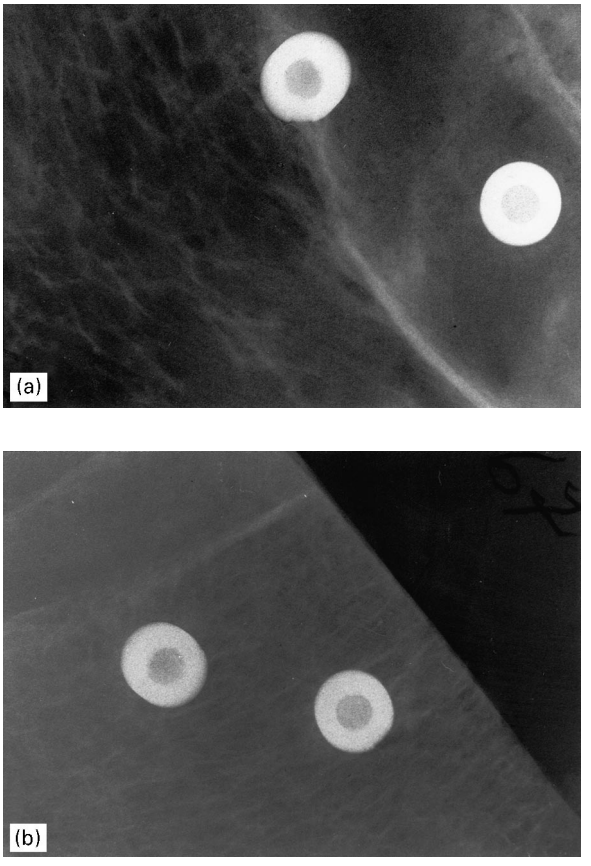


Figure 2 (a) Radiograph of two implants located in the canalis mandibularis, taken perpendicular to the long axis of the implant. (b) Radiograph of two implants surrounded by trabecular bone, in the vicinity of the canalis mandibularis.

alcohol series and finally embedded in methyl methacrylate. Thin undecalcified histological sections of approximately 10 μm thickness were produced with a modified diamond blade microtome [21, 22]. The sections were stained with methylene blue and basic fuchsin and were sawn in a horizontal plane, perpendicular to the long axis of the implant.

2.4. Histological evaluation

Histological and histomorphometrical evaluations were performed to evaluate the cortical and trabecular bone response to the implants.

The qualitative histological analysis consisted of a thorough description of the observed bone response.

First, for the histomorphometrical analysis the percentage of bone contact was measured on four sections of each implant using a light microscope connected to a computer equipped with a video and image analysis system (Technical Command Language image). Two of these sections were representative for the cortical bone reaction and two for the trabecular bone reaction (Fig. 3). The amount of bone-implant contact was measured for the total implant perimeter. Finally, the percentage of bone contact, defined as the length of the interfacial area with direct bone-implant apposition, was calculated.

Second, the amount of bone in two circular regions of interest around the implant was measured (Fig. 4). These measurements were performed using a stereomicroscope, which was connected to a video-camera. With the use of a frame grabber with 512×512 pixels, 8 bit grey-level images were captured. One of the two regions was defined in direct contact with the implant, at a radial distance of 0.265 mm from the interface (circle A). The other region was determined at 0.61 mm from the implant (circle B). Finally, the amount of bone in the area confined by circle A and in an area C (C corresponds to the amount of bone inside circle B subtracted from the amount of bone inside circle A) were calculated using the above-mentioned image analysis program. The amount of bone was quantified as 10^{-3} bone amount per square micrometre. This measurement was performed on the same implant sections as used for the bone contact evaluation.

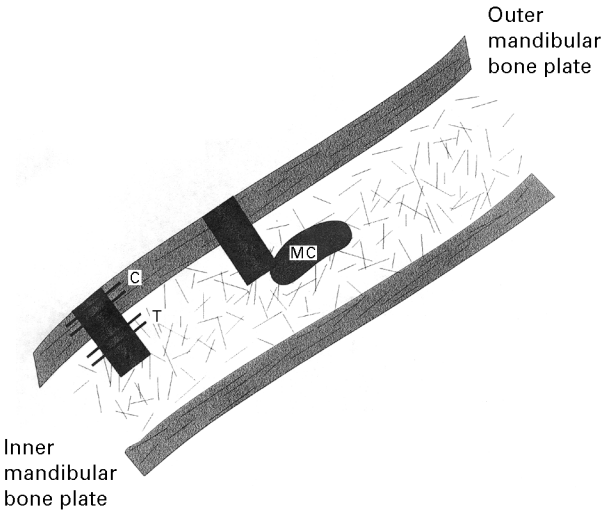


Figure 3 Schematic drawing showing that two histological sections were located in the cortical bone, C, of the outer mandibular bone plate, while two sections are located into the trabecular bone, T. It is also visible how the implants, I, are installed in the vicinity of the mandibular canal, MC.

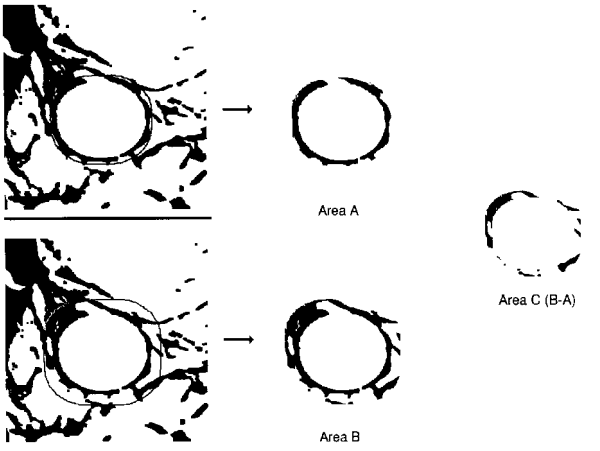


Figure 4 Drawing of the regions of interest used for the bone mass measurements. Circle A, region in direct contact with the implant, at a radial distance of 0.26 mm from the interface; circle B, region determined at 0.61 mm from the implant; circle C, amount of bone inside circle B subtracted from the amount of bone inside circle A.

3. Results

One goat had to be sacrificed 9 days after surgery because of a broken rib caused during her stay on the farm. The other 11 goats healed uneventfully. At sacrifice, no clinical signs of inflammation or adverse tissue reaction could be seen around the implants. Radiographs, taken before embedding of the samples, showed that 20 of the 44 retrieved implants were located partially into the mandibular canal.

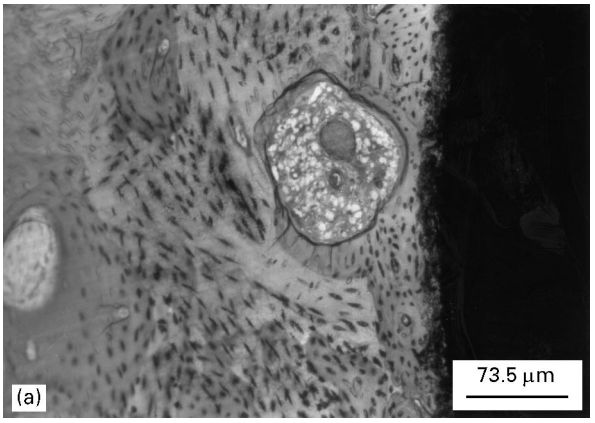
3.1. Descriptive histological evaluation

3.1.1. Implants located out of the mandibular canal

At the cortical bone level around all different implants, mature bone was observed. This bone was closely apposed to the implant surface without any intervening fibrous tissue interface (Fig. 5a).

The trabecular bone reaction was almost identical. Around all implants the trabecular bone showed frequently an intimate bone-implant contact (Fig. 5b). Occasionally, even the implants were completely surrounded by trabecular bone. In areas of bone contact, remodelling lacunae with osteoblasts were present.

For both the trabecular and the cortical bone, all coatings showed reduction in thickness. This coating reduction was not uniform; in some areas there was no coating left, while in other areas the coating did not disappear. Nevertheless, the HA coating reduction was most severe, while the HAHT coating showed a moderate reduction. The FA coating appeared to be very stable.



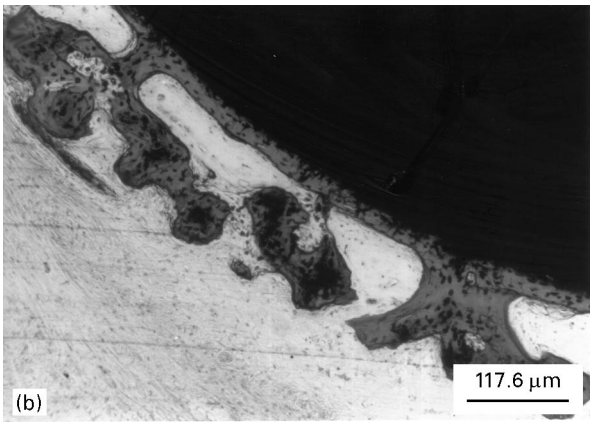
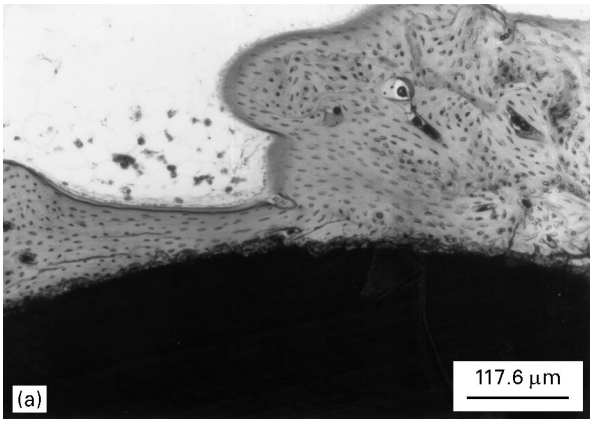


Figure 5 Photograph of two implants installed out of the mandibular canal, surrounded by (a) cortical and (b) trabecular bone.



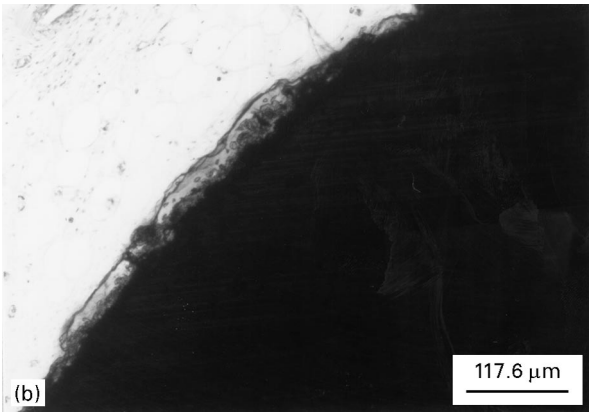


Figure 6 Photograph of two implants located in the mandibular canal, surrounded by (a) cortical and (b) trabecular bone.

3.1.2. Samples of implants in the mandibular canal

The cortical bone reaction to implants located in the mandibular canal was similar to the reaction to implants installed in a correct position outside the canal (Fig. 6a). Considering the malposition inside the canal, it was found that only some implants were inserted completely in the canal. Mostly, only a small part of the implant perimeter was in direct contact with the content of the canal. However, in both situations, no adverse tissue reactions were ever observed.

Considering the part of the implant located outside the canal, the trabecular bone response was similar to correctly placed implants (Fig. 6b). Further, only on some of the coated implants was a layer of osteoid and occasionally mature bone seen on the implant part located in the canal.

Evaluation of the coating reduction pattern revealed the same degree of decrease as the correctly installed implants.

3.2. Histomorphometrical evaluation

The implants that were partially located in the mandibular canal were excluded from the histomorphometrical measurements. The sections of the other implants were further evaluated.

3.2.1. Percentage of bone contact

All cortical and trabecular bone apposition data for the various implant surfaces are given in Tables I and II. Statistical testing, using a one-way analysis of variance (ANOVA) and a Tukey multiple-comparison procedure revealed no significant difference between the percentages of bone contact of the different Ca–P-coated and uncoated implants, nor between the percentages of bone contact of the different Ca–P-coated implants.

TABLE I Percentages of bone contact for cortical bone, where n is the number of evaluated implants

Material	Mean amount of bone contact ± standard deviation (%)
Ti	55.4 ± 15.6 ($n = 2$)
HA	64.3 ± 19 ($n = 7$)
HAHT	63.6 ± 15.5 ($n = 5$)
FA	77.9 ± 14.5 ($n = 6$)

TABLE II Percentages of bone contact for trabecular bone, where n is the number of evaluated implants

Material	Mean amount of bone contact ± standard deviation (%)
Ti	55.7 ± 22.6 ($n = 2$)
HA	60.9 ± 18.1 ($n = 7$)
HAHT	65.5 ± 18.5 ($n = 6$)
FA	71.6 ± 16 ($n = 6$)

3.2.2. Bone amount

In Table III the results are given of the bone amount measurements in areas A and C around the different

TABLE III Results of bone amount measurements

Coating	Amount of cortical bone (10^{-3} μm^2)				Amount of trabecular bone (10^{-3} μm^2)			
	Area A	Standard	Area C	Standard	Area A	Standard	Area C	Standard
Ti	9636.0	9900.9	4148.5	272.2	1143.5	733.3	1445.5	618.7
HA	5979.4	3717.3	4514.6	759.0	3248.4	3358.0	2413.3	911.3
HAHT	3047.4	303.7	4092.2	787.9	2394.2	354.1	3045.3	476.2
FA	8308.8	7347.1	4292.8	2505.8	1420.5	780.5	1422.0	918.3

implant types. The ANOVA and Tukey multiple-comparison test revealed no significant differences between the cortical and trabecular bone amount for the uncoated implants and that for the different coated materials.

4. Discussion

Load-bearing conditions can influence the bone reaction to different implant materials. Therefore, in this study a mainly load-free experimental design was chosen to investigate the bone response to different calcium-phosphate-coated and uncoated titanium implants. The intervening healing period clinically used after installation of oral implants formed the basis for choosing 12 weeks as the evaluation time. Radiological and histological investigation revealed that almost half the installed implants were located partially in the mandibular canal. It might be asked why we persisted to insert all implants when the surgical procedure was unsuccessful. However, reliable post-surgical radiographs to confirm the position of the implants could not be taken because of anatomical restrictions. Further, the standardized positioning of the implants was based on pilot studies done on cadavers. Apparently, there was no indication to suppose that the implants were not placed correctly. Consequently, the mandibular corner seems to be a very unsuitable and inconsistent animal model for investigating the bone response to implant materials. To prevent the influence of malpositioning on the final biological evaluation, all implants located in the mandibular canal were excluded from the histomorphometrical analysis. As a consequence, this resulted in inconsistency in the number of specimens for each implant material finally used in the statistical testing procedures.

Considering the cortical bone behaviour, the results are very consistent with several other studies performed by Jansen *et al.* [23–25] on rabbits. The data suggest that Ca–P coatings improve the cortical bone contact but statistical testing reveals that this difference is never significant. This phenomenon is due to the relatively high values of the standard deviation, which are probably related to differences between the cortical bone qualities of the various animals.

The histomorphometrical measurements also did not show significant differences between trabecular bone reactions of Ca–P-coated implants and uncoated implants. This observation is very surprising compared with our earlier study using the same implants but installed in the femoral condyle of goats [5]. In that study, we found a significantly lower percentage of trabecular bone in contact with uncoated cylindrical titanium implants, compared with Ca–P-coated cylindrical implants.

There are two hypothetical explanations for this finding. First, in the femoral condyle model, as shown by Heimke *et al.* [14], mechanical factors influence the remodelling process at the bone–implant interface. In the loaded conditions of that model the surface topographical characteristics of the implant play an important role. On the other hand, we know that the surface topographies of Ca–P-coated and uncoated grit-blasted titanium implants are not completely the same. Consequently, in mainly load-free conditions as in the distal part of the mandible, the influence of the surface topography will be negligible, as long as there are no mechanical forces acting on the implant. A second explanation for the difference between the bone responses of implants inserted in the femoral condyle and the mandibular corner could be the difference between the bone morphologies. The mandibular bone consists of a thin layer of trabecular bone surrounded by dense cortical bone. The femoral condyle consists mainly of trabecular bone; only a very thin layer of cortical bone is present. The composition of mandibular bone will result in a better initial stability of implants, independent of their surface morphology. In addition, it has to be noted that the femoral trabecular bone has a spongy appearance, while the mandibular trabecular bone has a lamellar appearance (Fig. 7).

Histological evaluation of the "failed" implants showed that only on the surface of some Ca–P-coated implants was bone present on the perimeter section located in the mandibular canal, while bone was never observed on the uncoated implants. We suppose that this bone originates from the bone wall surrounding the canal and has migrated subsequently over the implant surface. Nevertheless, this observation proves again the osteoconductive properties of Ca–P coatings [26], providing a scaffold for new bone growth.

The same findings for the coating reduction were observed as in our previous studies [5, 27]; the coating reduction did not influence the bone behaviour in this 12 week study as we saw no significant differences between the bone reactions of the various coatings. The histological sections showed that, even on places where the coating completely disappeared, the bone

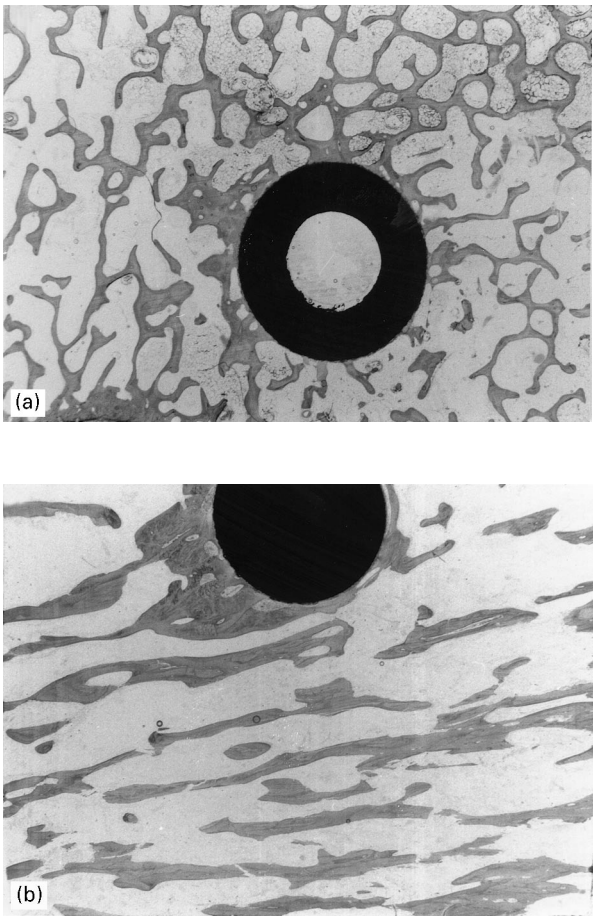


Figure 7 Two photographs of a cylindrical implant inserted in the trabecular bone (a) of the femoral condyle and (b) of the mandible showing the difference in bone structure between these two different skeletal parts: (a) spongy appearance of the trabecular femoral bone; (b) lamellar structure of the trabecular mandibular bone.

was in close contact with the implant surface. Because of the similar observations to previous studies, the coating reduction was not quantified in the present study.

In summary, this study shows that the distal corner of the mandible is not an optimal model for installation of implants because of anatomical restrictions. Further, the experiment remained inconclusive with respect to the influence of loading conditions on the bone behaviour, probably because of morphological differences between the bone structures of various skeletal parts. Despite these observed problems, the histological results confirmed at least the bioactive properties of Ca–P coatings.

Acknowledgements

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