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Assessment of bone surrounding implants in goats: ex vivo measurements by dual X-ray absorptiometry

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The aim of the present study is to determine the possibility of measuring the bone mineral density (BMD) around implants by dual energy X-ray absorptiometry (DEXA). Therefore, the trabecular BMD was measured close to 127–600 μm and at a distance from various uncoated and Ca–P-coated implants inserted into the femoral condyle of goats. The implants were left in situ for 12 weeks. In addition, the bone–implant interface was evaluated histologically. For comparative reasons the BMD of non-implanted lateral and medial femoral condyles was also measured.

The reproducibility of the measurements, expressed as a coefficient of variation, was found to be 0.44%. Moreover, the regions closest to the implants exhibited a higher BMD than all other regions, and the regions located in the medial condyle showed a higher BMD than the lateral condylar regions. Although the histological sections of the implants in the medial condyle demonstrated more bone contact with the coated than with the uncoated implants, a higher density was measured around the uncoated implants. The results regarding the non-implanted condyles indicated a higher density in the medial than in the lateral condyle. In view of these results, we conclude that BMD around dental implants depends on the location of the implant and that DEXA appears to be an excellent tool for analysing bone–implant reactions. © 1997 Elsevier Science Limited. All rights reserved

Keywords: Dual energy X-ray absorptiometry (DEXA), bone mineral density, trabecular bone, implants, dual photon absorptiometry (X-photon)

Received 5 October 1995; accepted 22 September 1996

Clinical survival percentages of implants have been clearly associated with the quality of the bony environment that surrounds the implants1,2. In addition to this, it is known that the use of calcium phosphate (Ca–P) ceramics for the manufacturing of implants can result in an improved bone response3. The exact mechanism underlying this biological advantage of Ca–P ceramics is not yet completely understood.

In most of the studies of bone growth around oral implants, histology is used for the assessment of the bone reaction. However, a less aggressive technique would be more appropriate. This would make it possible to acquire information not only about the final quality of the newly formed bone but also during the course of the healing response.

Besides histological appearance, an important measure of bone quality is the bone mineral density (BMD)4. However, according to Keratli et al.5 BMD cannot be reliably determined by means of conventional techniques. For example, using radiographs, deviations in BMD of more than 30% are observed. In contrast, another method that has been reported to be more reliable for evaluating the bone mineral status of skeletal bone is dual energy X-ray absorptiometry (DEXA)6–10. In several studies the efficacy of this technique for the measurement of bone condition changes into porous implant materials11 and at defined distant areas around femoral hip implants has been demonstrated6,12,13. Although this approach is very suited to the overall monitoring of implant behaviour, quantification of the BMD within a distinct narrow zone close to the implant will provide more predictable information about the actual bone response. Therefore, supported by recent software improvements for quantifying BMD, the aim of the present study was to determine the applicability of the DEXA technique to the examination of the influence of implant materials on the interfacial bone healing capacity. In vitro BMDs were measured directly adjacent to and at various
distances from various Ca-P coated implants inserted into the trabecular bone of the femoral condyles of goats. In addition, information about the influence of the Ca-P ceramics on the trabecular bone response was obtained. Further, to determine whether BMD measurements are indeed predictive of bone behaviour around implants, the results were compared with the additionally obtained histological and histomorphometrical data.

MATERIALS AND METHODS

Implants

Twelve healthy, adult, female goats, with an average age of 30 months and a weight of 50-80 kg, provided the model material. The animals were kept in quarantine for at least 4 weeks and tested for CAE/CL-arthritis.

For the experiment, cylindrical TiAl6V4 (Ti) implants were manufactured, measuring 10 mm in length. The implants were at one side provided with a central cylindrical opening. All implants were grit-blasted with Al2O3 (Ra = 4-5 μm). They were cleaned ultrasonically in propanol and dried at 100°C. Subsequently, they were left either uncoated or coated with a Ca-P film, approximate thickness 55-60 μm, using a plasma-spray process. Three different coatings were used:

1. Hydroxyapatite coating with a crystallinity of 60% (HA).
2. Hydroxyapatite coating subjected to heat treatment (650°C for 10 min), resulting in a slight increase of the crystallinity to 65% (HAHT).
3. Fluorapatite coating with a crystallinity of 95% (FA).

The final diameter of the coated and uncoated implants was 4 mm. The coatings were characterized by X-ray diffraction (XRD) and infrared (IR) spectroscopy.

Prior to surgery, all implants were cleaned ultrasonically in 100% ethanol to remove any loose particles and dried at 50°C. Finally, the implants were sterilized in an autoclave.

Animal surgery

The implants were placed into the trabecular bone of the femoral condyle. Each animal received four implants, in the lateral and medial condyles of both the left and right femora. Anaesthesia was induced by intravenous pentobarbital, 25 mg kg⁻¹, and each animal was also given 0.5 mg of atropine. After orotracheal intubation, anaesthesia was maintained by ethrane solution, 2-3%, through a constant volume ventilator. For the insertion of the implants, a longitudinal incision was made in the medial and lateral surfaces of both the left-side and right-side femora, after shaving and disinfecting the operation area with povidone-iodine. After exposure of the femoral condyle, pilot holes were drilled in the trabecular bone. These holes were gradually widened to the final diameter of the implants. The bone preparation was performed with a very gentle surgical technique and with continuous internal cooling. Following the press-fit insertion of the implants, the soft tissues were closed by separate layers, using resorbable vicryl 2-0 sutures. For prophylaxis, antibiotic Albipen was administered for 3 days, starting 1 h postoperatively. A total of 48 implants were placed: 12 Ti, 12 HA-coated, 12 HAHT-coated and 12 FA-coated implants. The implants were placed according to a balanced split-plot design. Three months after insertion of the implants, the animals were killed with an overdose of Nembutal. The femoral condyles were excised and preserved in a buffered 10% formalin solution.

Preparation for dual X-ray absorptiometry

After fixation, the specimens were dehydrated by an alcohol series. For standardized orientation during sawing and scanning, one part of a wooden cocktail stick was placed into the cylindrical opening of each plant and the other part was situated outside the implant (Figure 1). Finally, the specimens were embedded in polymethylmethacrylate (PMMA), a tissue-equivalent material. Using the Conrad sawing-device, the specimens were sectioned parallel to the long axis of the cocktail stick to a final thickness of 13 mm. The average surface area of the section was 2 cm x 3 cm (Figure 1). The sawing section was chosen in such a way that access of the X-ray beam to the trabecular bone structure was possible in the DEXA procedure.

For comparative reasons the medial and lateral parts of the femoral epicondyle of untreated goats also had to be measured. For this part of the study, we used the...
Figure 2 Scan of the non-implanted sliced femoral epicondyle of goats A, B and C. The regions of interest A1 and A2 are respectively situated in the lateral and medial parts.

Excised left- and right-side femoral epicondyles of three female goats (A, B and C). These condyles were sectioned in the sagittal plane. Similar to the implant, the specimens were 13 mm thick (Figure 2).

Dual X-ray absorptiometry scanning procedures

The DEXA measurements were made utilizing a Hologic QDR-1000 bone densitometer (Hologic Inc., Waltham, MA, USA). We used a source collimator of diameter 1 mm and high-resolution software. The equipment was calibrated daily with a lumbar spine phantom on calcium oxhydroxyapatite. The coefficient of variation of precision is 1.0 for lumbar disc, 1.6 for femoral neck and 1.5 for trochanter major. The line spacing and point resolution were both 0.0127 cm. To define the BMD around and at various distances from the implants, seven regions (R1, R2, ..., R7) of interest were measured. These regions were situated in the trabecular bone structure parallel to the long axis of the implant (Figure 3). Region R1 was close to the implant at a distance of 0.0127 cm, region R2 was adjacent to the first region, and then immediately followed by region R3. To compare these regions with bone density at a distance from the implants, in the same sequence, control regions R4, R5 and R6 were positioned anywhere in the condyle. Region 7 was constantly situated at a distance of 16 pixels cranial to the implant. Each region had a length of 45 pixels and a width of 5 pixels (1 pixel = 125 μm). Each sawn-off block was scanned five times using the autoscan program. The scans were analysed twice with the regions R1, R2 and R3 all caudal (Figure 3) to the implant and then cranial (Figure 4) to the implant. The other regions were kept at their original positions in the second series of the analysis.

To make inquiries about the BMD in the total area of the six untreated control blocks from three animals without any implants we had to use the lumbar spine software with variable resolution and a collimator of 2.3 mm, because of the size of the sliced epicondyle. These measurements were performed using the Hologic bone densitometer. The line spacing was 0.0470 cm and the point resolution was 0.0481 cm. The sizes of the lateral region A1 and the medial region A2 were both 69–47 pixels (Figure 2). The samples were immersed in a tissue-equivalent material (5 cm deep water). To determine the differences in density in the central direction (see arrows in Figure 5) as well as in the cranial direction (see arrows in Figure 6) of the sliced epicondyle, we used the regions P1–P6, sized 7 × 27 pixels (Figures 5 and 6). BMD, as measured by DEXA, is usually expressed as grams per surface area (g cm⁻²). This definition of ‘density’ has been used by the manufacturer because it consists of a projected BMD of cortical as well as trabecular bone. In the present investigation, all measurements were

Figure 3 Scan of the sawn-off block containing an implant. The regions of interest, R1–R3, are situated caudal to the implant. Region R7 is at a distance of 16 pixels. R4–R6 are positioned anywhere in the condyle.

Figure 4 As in Figure 3, but R1–R3 are now situated cranial to the implant.
performed only in slices of trabecular bone, with a thickness of 13 mm. Consequently, density was defined as grams per volume unit (g cm⁻³).

**Histological procedures**

After scanning the blocks, thin (10 μm) sections were prepared using a modified diamond (blade sawing) micromtome technique. The sections were made perpendicular to the long axis of the implants. These sections were stained with methyl blue and basic fuchsin and examined by light microscopy. To define the percentage of bone contact, the microscopic images were projected onto a monitor, using a video camera coupled to the light microscope. The percentage of bone contact was defined as the length of the interfacial area where there was direct bone apposition divided by the total implant perimeter multiplied by 100. Three sections were used from each implant for bone contact analysis. The results presented are based on the average value of these three measurements.

**RESULTS**

All animals recovered quickly. One goat had to be excluded 9 days after surgery because of a broken rib. The other animals did not show any clinical signs of inflammation or adverse tissue reaction around the implants. Consequently, at the end of the implantation period 44 implant-containing specimens were present for evaluation.

Scans taken perpendicular to the long axis of the implant showed that the implants were actually located in the trabecular bone. Only their coronary parts were surrounded by cortical bone (Figure 7).

**Precision of the measurements**

The BMD was calculated as bone mineral content in g cm⁻³. By measuring every block five times, the precision was expressed as the coefficient of variation for every region. The averages of these coefficients of variation for regions R1–R3 (close to the implants) were, respectively, 0.44, 0.40 and 0.40%. For the regions at distance from the implants (R4–R7), the coefficients of variation were, respectively, 0.49, 0.43, 0.47 and 0.44%.

**BMD by implant location**

Statistical analysis by Student’s t-test showed, for both implants inserted in the medial and lateral condyles significantly higher BMD of region R1 compared to regions R2–R7 (P < 0.001); see Table 1. The BMDs of regions R1, R2, R3 and R7 located in the medial side of the condyle exhibited a higher bone density compared to the corresponding regions in the lateral side. However, regions R4–R6 showed the reverse.

**BMD by implant type**

Table 2 shows the BMD for region R1 related to implant type and location. Statistical analysis, using the analysis of variance and the Newman–Keuls multiple comparison procedure, demonstrated that BMD for region R1 of the various implants was significantly (P < 0.001) higher in the medial than in the lateral...
Table 1 Means, standard deviations and ranges of the bone mineral densities (BMDs) in regions R1–R7 for location in 44 implants

<table>
<thead>
<tr>
<th>Region</th>
<th>BMD (g cm⁻³)</th>
<th>Medial</th>
<th>Lateral</th>
</tr>
</thead>
<tbody>
<tr>
<td>R1</td>
<td>0.6369 ± 0.09</td>
<td>0.5882 ± 0.08</td>
<td></td>
</tr>
<tr>
<td>R2</td>
<td>0.6146 ± 0.09</td>
<td>0.5679 ± 0.08</td>
<td></td>
</tr>
<tr>
<td>R3</td>
<td>0.6122 ± 0.09</td>
<td>0.5665 ± 0.09</td>
<td></td>
</tr>
<tr>
<td>R4</td>
<td>0.5405 ± 0.12</td>
<td>0.5627 ± 0.09</td>
<td></td>
</tr>
<tr>
<td>R5</td>
<td>0.5477 ± 0.12</td>
<td>0.5655 ± 0.09</td>
<td></td>
</tr>
<tr>
<td>R6</td>
<td>0.5543 ± 0.12</td>
<td>0.5648 ± 0.09</td>
<td></td>
</tr>
<tr>
<td>R7</td>
<td>0.5788 ± 0.09</td>
<td>0.5620 ± 0.08</td>
<td></td>
</tr>
<tr>
<td>Range</td>
<td>0.8520–0.27</td>
<td>0.8340–0.37</td>
<td></td>
</tr>
</tbody>
</table>

*The number of measurements per region was 5 (N = 5).

Table 2 Means, standard deviations and ranges of bone mineral densities (BMDs) for region R1, pertaining to the various implants and locations

<table>
<thead>
<tr>
<th>Implant</th>
<th>BMD (g cm⁻³)</th>
<th>Medial</th>
<th>Lateral</th>
</tr>
</thead>
<tbody>
<tr>
<td>FA</td>
<td>0.6024 ± 0.07</td>
<td>0.5854 ± 0.04</td>
<td></td>
</tr>
<tr>
<td>HAHT</td>
<td>0.5697 ± 0.07</td>
<td>0.5797 ± 0.12</td>
<td></td>
</tr>
<tr>
<td>HA</td>
<td>0.6326 ± 0.09</td>
<td>0.6016 ± 0.08</td>
<td></td>
</tr>
<tr>
<td>Ti</td>
<td>0.7050 ± 0.06</td>
<td>0.5784 ± 0.05</td>
<td></td>
</tr>
<tr>
<td>Range</td>
<td>0.4569–0.88</td>
<td>0.3905–0.93</td>
<td></td>
</tr>
</tbody>
</table>

Table 3 Bone mineral densities (BMD) for the medial and lateral parts of the left (L) and right (R) sectioned femoral epicondyles of goats A, B and C

<table>
<thead>
<tr>
<th>Goat epicondyle</th>
<th>BMD (g cm⁻³)</th>
<th>Medial</th>
<th>Lateral</th>
</tr>
</thead>
<tbody>
<tr>
<td>AL</td>
<td>0.503</td>
<td>0.432</td>
<td></td>
</tr>
<tr>
<td>AR</td>
<td>0.541</td>
<td>0.447</td>
<td></td>
</tr>
<tr>
<td>BL</td>
<td>0.566</td>
<td>0.463</td>
<td></td>
</tr>
<tr>
<td>BR</td>
<td>0.532</td>
<td>0.477</td>
<td></td>
</tr>
<tr>
<td>CL</td>
<td>0.664</td>
<td>0.469</td>
<td></td>
</tr>
<tr>
<td>CR</td>
<td>0.602</td>
<td>0.489</td>
<td></td>
</tr>
</tbody>
</table>

Table 4 Means and standard deviations of bone apposition (%) for the various implants for the two implantation sites

<table>
<thead>
<tr>
<th>Implant</th>
<th>FA</th>
<th>HAHT</th>
<th>HA</th>
<th>Ti</th>
</tr>
</thead>
<tbody>
<tr>
<td>Medial</td>
<td>82.36 ± 7.2</td>
<td>80.58 ± 10.7</td>
<td>84.34 ± 5.5</td>
<td>60.82 ± 12.7</td>
</tr>
<tr>
<td>Lateral</td>
<td>72.8 ± 10</td>
<td>76.98 ± 8.5</td>
<td>74.03 ± 11</td>
<td>49.34 ± 21.4</td>
</tr>
<tr>
<td>Mean</td>
<td>78.4 ± 9.1</td>
<td>79.1 ± 8.2</td>
<td>78.2 ± 9.4</td>
<td>56.8 ± 16.9</td>
</tr>
</tbody>
</table>

*The number of implants in each group was 11.

Bone contact between implants inserted in medial and lateral condyles (P < 0.005). The bone contact of the Ca–P-coated implants was also significantly (P < 0.001) higher in comparison with the uncoated implants. No significant difference existed between the FA-, HA- and HAHT-coated implants.

DISCUSSION

The repeated analysis showed that the precision of the measurements, with coefficients of variation ranging from 0.40 to 0.44% for regions R1–R3 and from 0.44 to 0.49 for regions R4–R7, is satisfactory.

The precision of the measurements in the excised untreated epicondyles of goats is very similar to previous studies, using lumbar spine protocol, by Kaymakci and Wark14 and Corten et al.15. They found a precision of about 0.5%.

Considering the BMD by location, the huge differences between the standard deviations are most probably caused by the extremely large range in density between the regions. In addition, it is observed that the medial regions 1, 2 and 3 show a significantly higher density compared to the lateral regions 1, 2 and 3. The reference regions 4, 5 and 6 show the opposite. These regions are, due to space limitations at both sides of the implants, placed towards the centre of the epicondyle compared to regions 1, 2 and 3. This phenomenon can be explained by the results of the BMD measurements in the untreated epicondyles. In these arbitrarily chosen epicondyles, the BMD in the medial compartment was also different from the lateral compartment. This difference is probably due to a difference in load bearing, which influences bone turnover. Studies by Seballe3 of implants placed in the epicondyle of Labrador dogs showed a similar pattern of distribution of bone density. Consequently, it can be concluded that the BMD around implants is dependent on the location of the implant. This conclusion is supported by the histological findings showing that the bone contact around the implants demonstrated a variation up to 20% in the amount of bone contact between the three sections of each implant used. Table 4 shows all percentages of bone contact for the various implants and implantation sites. One-way analysis of variance (ANOVA) and a multiple comparison procedure (Newman–Keuls) revealed a significant difference in bone contact between implants inserted in medial and lateral condyles (P < 0.005). The bone contact of the Ca–P-coated implants was also significantly (P < 0.001) higher in comparison with the uncoated implants. No significant difference existed between the FA-, HA- and HAHT-coated implants.
amount of bone apposition was also significantly higher near the implants inserted in the medial compared to those in the lateral sides. The combination of these findings again confirms that, besides the original bone quality and used implant material, the amount of bone apposition to implants is also determined by biomechanical forces.

It should also be noted that the observed higher densities in all regions (R1) close to the implants indicate that there is a strong remodelling activity at distances of approximately 127 up to 600 μm (1–5 pixels) from the implants. This phenomenon could result from surgery. Effects are still discernible 3 months after implantation. On the other hand, the possibility of a persisting bone reaction influenced by the type of implant material cannot be excluded. This
hypothesis is supported by the lower percentage of bone apposition to the uncoated Ti implant and the higher bone density in region R1 around these implants inserted in the medial epicondyle. The final bone reaction to an implant will be determined by the degree of compatibility and integration of the inserted implant under certain defined conditions. Consequently, a less than ideal bone biocompatibility will result in a reduced integration of the implant in the stress-transferring system of the surrounding bone trabeculae. This implies that, similar to the soft tissue situation, the implant will act as a constant mechanical stimulus. This trauma will result not only in fibrous encapsulation of the implant but also in more bone turnover around the implant.

CONCLUSIONS

The present study shows that two variables, the implant biocompatibility and the loading conditions, play a primary role in the amount of bone apposition around implants. Further, we conclude that, in addition to histological analysis, dual energy X-ray absorptiometer appears to be an excellent tool in establishing this reaction. Consequently, these results stress the need for more research and the development of a device for in vivo clinical measurements in (implant) dentistry.

REFERENCES