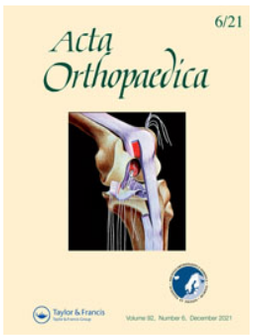

Acta Orthopaedica Scandinavica

ISSN: 0001-6470 (Print) (Online) Journal homepage: informahealthcare.com/journals/iort19

Invasion of bone into porous fiber metal implants in cats

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To cite this article: Helge Rønningen, Ludvig Fjeld Solhelm & Norvald Langeland (1984) Invasion of bone into porous fiber metal implants in cats, Acta Orthopaedica Scandinavica, 55:3, 352-358, DOI: 10.3109/17453678408992373

To link to this article: https://doi.org/10.3109/17453678408992373

Published online: 08 Jul 2009.

Acta Orthop Scand 55, 352–358, 1983

Invasion of bone into porous fiber metal implants in cats

Bone ingrowth in porous Ti-6Al-4V implants in feline femurs was assessed for the amount and composition 4, 12 and 26 weeks after implantation. During the first 12 weeks there was a rapid ingrowth that levelled out during the ensuing weeks. Bone had penetrated the implants to an average depth of 0.5 mm over their entire surface at 26 weeks. The ingrown bone was deficiently mineralized at 4 and 12 weeks, but reached full mineralization at 26 weeks. A low calcium to phosphorus ratio persisted at all observation times. The ingrown tissue was compared to bone filling drill holes that had been made in the trochanters contralaterally to the implants. By means of radioactive strontium, the mineral deposits in the holes and implants could be compared. The overall results indicate that bone ingrowth in a porous implant and bone healing are similar processes both qualitatively and quantitatively.

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Failures in long-term fixation of weight-bearing implants in orthopaedic surgery have stimulated frantic research into new alternatives for fixation of these devices. Implants featuring a porous surface for fixation by bone ingrowth come into this category, and a variety of porous materials have been introduced (Klawitter & Hulbert 1971, Galante & Rostoker 1973, Collier et al. 1976, Robertson et al. 1976, Clarke et al. 1979, Ehrnford et al. 1980, Martens et al. 1980, Tuinzing & Swart 1981, Rhinelander et al. 1982, Lord 1982). Galante & Rostoker (1973) and Rostoker et al. (1974) have developed a porous implant made from titanium wire (fiber metal). This implant has completely interconnected pores, suitably sized for bone ingrowth. Eventually, all the void spaces are filled by bone, giving the impression that the implants are integrated parts of the bones (Andersson et al. 1978). Long-term studies in baboons have demonstrated that bone can host these implants for at least 10 years without any detectable tissue side effects (Memoli et al. 1983). In a recent study in dogs it was found that the invading bone remodels from an immature appearance 3 weeks after implantation to lamellar bone at 7 weeks (Rønningen et al. 1983). This is very similar to the pattern described for healing of long bones in dogs (Anderson 1967).

Our study was designed to analyse the composition of bone invading the fiber metal compared to normal bone and bone being formed in a standardized bone defect. A chemical approach was chosen as it allows a more direct quantification than do histological methods.

Material and method

Implants

Cylindrical implants made from the Ti-6Al-4V alloy (ninety per cent titanium, 6 per cent aluminium and 4 per cent vanadium; kindly supplied by Dr. William Rostoker, University of Illinois, Chicago, USA) were used. The dimensions were 4.8 mm diameter by 9.0 mm height. The implants had been manufactured by compressing 0.25 mm thick wire stumps to a porous structure of 50 per cent metal and 50 per cent void space (Rostoker et al. 1974).

Experimental procedure

Nineteen male adult cats (*felis domestica*), weighing 3.0–5.0 kg, were used. General anaesthesia was induced by means of xylacine chloride 20 mg i.m. (Rompun Vet®, Bayer) followed by pentobarbitalum 40–80 mg i.v. (Mebumal Vet 5%®, Rikshospitalets Apotek, Oslo). Each animal underwent the following

procedure: Drill holes were made from the lateral side in both proximal femurs using a 4.7 mm (outer diameter) drill bit directed perpendicularly to the long axis of the bone and centered at the level of the lesser trochanter. The drill was advanced to the medial cortex. The holes were vigorously irrigated with saline to remove all bone debris. Implants were placed in the left side holes with their end surfaces flush with the periosteum. The slightly undersized holes allowed primary fixation by means of press fit. The right side holes were left empty. The procedure was performed under aseptic conditions. Benzylpenicillin 0.25 g and dihydrostreptomycin 0.25 g (Proca-Mycin Vet®, Apotekernes Laboratorium, Oslo) were given i.m. on the day of surgery and the next 2 days. Six animals were killed 4 weeks postoperatively, six at 12 weeks and seven at 26 weeks. The day before killing, the animals were anaesthetized, and labelling with 85-strontium (NEN Chemicals, W. Germany) was used as a tracer of bone mineral. Activities of 1.5–3.5 MBq kg^{-1} were administered through a cephalic vein. As the anaesthesia was continued, blood samples were drawn from a femoral vein 1, 2, 4 and 8 h after labelling to obtain data for serum radioactivity. The following day a second anaesthesia was given. A blood sample was drawn and the proximal halves of both femora and the proximal third of the right tibia were removed 24 h after radiolabelling. After this, the anaesthetized animals were used in an experiment in audiophysics (unrelated to the bone experiment) before a final blood sample was drawn and the animals were killed (26–31 h after labelling).

Analyses

The femurs were radiographed to check the positions of the holes and implants. After 1 week of dehydration in acetone, the bone surrounding the implants was removed mechanically to leave only the bone within the pores for analysis. The tissue that had grown into the drill holes was removed by means of a trephine with an inner diameter of 5.2 mm. For the 4 weeks interval this could be done directly, guided by a visible dimple in the cortex where the hole had been. For the longer intervals the dimple was fainter, requiring radiographs and the lesser trochanter to locate the hole correctly. The tibias were mounted in a miter box and two standardized 7.5 mm thick slices cut from the metaphysis. The proximal slices served as reference samples of cortical and cancellous bone. The cancellous bone was removed from the distal slices before the specific gravities of compact bone were determined using the Archimedes principle (Arnold 1960).

All bone samples were further dehydrated in acetone and dried in air to constant weight. The specific gravity of dry bone was used to estimate the average depth to which bone had penetrated the implants. The dry weights of the bone within the implant pores were found by subtracting the weight of the implant before surgery. The radioactivity of this tissue was measured in a Packard Auto-Gamma Scintillation Spectrometer. The tissue was then extracted from the implants in 2.00 ml 6 M hydrochloric acid for 24 h at room temperature. After this, the implants were washed twice in 1.00 ml 6 M hydrochloric acid. Recounting showed that the extraction and washing procedures had removed more than 99 per cent of the radioactive strontium from the implants. Finally, the implants were torn apart by force and inspected under high power (40×) magnification. This confirmed that no tissue was left on the fibers. Hence, it was considered that all tissue had been transferred from the implants to the 4.00 ml of hydrochloric acid. The hydrolysis was completed in sealed tubes at 125°C for 18 h. The tibia slices and tissues from the holes were put directly into 6 M hydrochloric acid and hydrolyzed in the same manner. The amount of collagen was determined by analysing the content of hydroxyproline, as described by Firschein (1969). The calcium and phosphorus contents were measured by a photometric method (Abbott Bicromatic Analyzer, Abbott Laboratories, USA). The calcium concentration in the serum was analysed by the same equipment. Aliquots of serum and bone hydrolysates were counted for amount of radioactive strontium. Total counts of 4000 or more were obtained for each aliquot. The serum activities were related to the serum calcium content as a specific radioactivity that was used in the further computations. A graphical analysis (Jacquez 1972) was applied in each animal to arrive at a mathematical expression where the specific activity of serum A(t) at time t after radiolabelling is a function of time. This can be written as a sum of exponentials of the general form:

$$1)\quad A(t) = a_1 \exp(-k_1 t) + a_2 \exp(-k_2 t) + \ldots + a_n \exp(-k_n t)$$

where $a_1, a_2, \ldots a_n$ and $k_1, k_2, \ldots, k_n$ are positive constants. The average specific activity in serum from time $t_0 = 0$ to $t_{24} = 24$ h when the bones were removed was computed as:

$$2)\quad \bar{A} = 1/24 \int_0^{24} A(t)\, dt$$

It was assumed that 85-strontium and calcium enter bone in the same ratio as the two elements exist in

blood (Cohn et al. 1963, Reeve & Hesp 1976). Hence the amount of calcium that arrives in a bone during a time interval was computed from the 85-Sr data by dividing the total radioactivity in the bone by the average specific serum activity during that particular interval (Bauer 1954). For Equation 1) to be valid, the computed A(t) should yield the same activities as those actually measured in serum. Hence the ratio between computed and measured activity should be 1. These ratios were computed for all serum samples (n = 114).

The ingrown tissue can maximally occupy the entire void space of an implant. According to this, we adopted the concepts of a restricted growth model to describe the ingrowth mathematically (Draper & Smith 1981). The contents of hydroxyproline and calcium of the ingrown tissue were expressed by the equation:

$$3) \quad X(t) = B\,(1 - \exp(-k\,t))$$

where B and k are positive constants determined by iterative fit towards least squares. B denotes the upper limit of X as t (weeks after implantation) tends to infinity. If B is set to 100 per cent, X will be expressed as a percentage of maximum, and the graph of Equation 3) will have 100 per cent as the upper limit. This transformation was performed to allow a direct comparison between hydroxyproline and calcium.

Statistics

If not otherwise stated, averages are expressed as the arithmetic mean, and dispersion as total range. Analysis of variance was used to test for differences between multiple means. Where differences were found, means were contrasted using *a posteriori* tests (Sokal & Rohlf 1969) Regression and correlation analyses were performed as described by Bhattacharyya & Johnson (1977) and Snedecor & Cochran (1967).

Results

All animals recovered well after surgery. At sacrifice all implants were found to be firmly fixed to the bone, without any sign of infection or metal-evoked tissue reaction. The radiographs showed all implants to be correctly seated without any translucent zone at the bone-implant interface (Figure 1). The average dry weights of the tissue that had grown into the implants were 32.9 mg (28.2–37.1) at 4 weeks, 53.3 mg (32.3–77.8) at 12 weeks and 52.8 mg (43.1–72.5) at 26 weeks. As the specific gravity of dry compact bone was found to be 1.64 ± 0.08 mg mm^{-3} (mean ± SD), 52.8 mg of ingrown tissue at 26 weeks means that the bone had penetrated the entire implant-surface to an estimated average depth of 0.45 mm. This is based on the following assumptions: exactly 50 per cent void space in the implants, and complete filling of the superficial pores by compact bone.

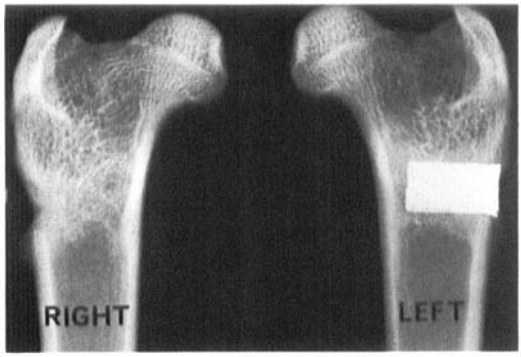

Figure 1. Proximal femora from a 4-week animal. The left femur has an implant in position. The right femur has a discontinuity in the lateral cortex where the hole was made.

For the implants the contents of hydroxyproline and calcium are shown in Table 1. Apparently most of the ingrowth had taken place before 12 weeks, to level out between 12 and 26 weeks.

The mineralization of the bone samples has been expressed as the molar ratio between calcium and hydroxyproline (Table 2). At 4 weeks, the bone in the implants was significantly undermineralized compared with the holes and tibias. At 12 weeks, the implant bone was deficiently mineralized compared with the holes.

Table 3 details the molar ratios between

Table 1. Constituents of the tissue in the porous implants (mol × 10^{-6}). Means and total ranges are given. The coefficients of variation ranged from 13 to 40 percent.

Interval	Hydroxyproline	Calcium
4 weeks	7.2 (5.1– 8.9)	114 (93.2–137)
12 weeks	11.7 (6.4–17.4)	216 (122–367)
26 weeks	11.2 (9.3–13.4)	226 (161–276)

Table 2. Mineralization of the bone samples expressed as the molar ratios between calcium and hydroxyproline. Means and total ranges are given.

Sample Weeks	Implants	Holes	Tibias
4 (n=6)	15.9** (14.6–18.4)	19.2 (17.9–20.5)	
12 (n=6)	18.3*** (15.6–21.1)	20.9 (19.7–22.3)	19.5* (16.1–23.1)
26 (n=7)	20.0 (17.3–21.4) (20.3–22.4)	21.5	

* Means for the three time intervals have been pooled in one group as there was no significant difference between the subgroups.
** Differ significantly from the corresponding hole values (19.2) and from the tibia values (19.5), ($p < 0.01$).
*** Significantly different from the corresponding hole molar ratio (20.9), ($p < 0.01$).

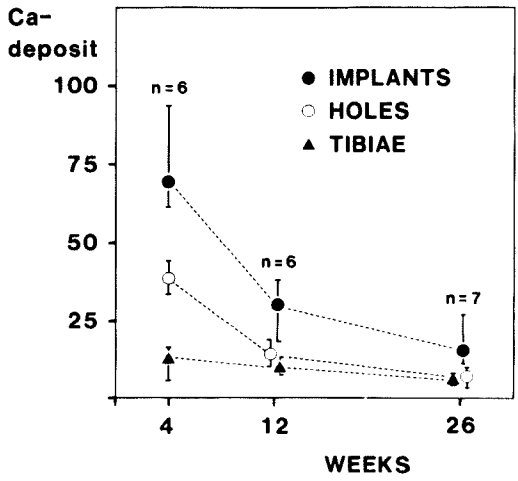

Figure 2. Mineral deposits calculated from isotope data. The deposits have been expressed as added amount of calcium ($mol \times 10^{-3}$) per amount of calcium (mol^{-1}) present in the tissue and 24 h (h^{-1}). Means and total ranges are given. The coefficients of variation ranged from 10 to 35 per cent.

calcium and phosphorus. At all intervals the ratios for the implants were significantly lower than for the corresponding holes and tibias.

The specific activities of the serum samples could be analysed as a two-compartment system yielding a two-term exponential function (Equation 1, n=2). The ratio between activities (computed by Equation 1) and measured activities was 1.00 ± 0.03 (mean ± SD).

The calculated deposit of new calcium per amount of calcium per day is shown in Figure 2. The deposits dropped rapidly from 4 to 12 weeks for both the implants and the holes, followed by a modest reduction during the ensuing interval. The values for the holes were uniformly lower than for the implants. But a marked parallellism between implants and holes in terms of change from interval to interval is visible. Hence a correlation analysis was performed to identify the relationship between the mineral deposit in the implants and in the holes. The correlation coefficient (Pearson) was

Table 3. Molar ratios between calcium and phosphorus. Means and total ranges are given.

Sample Weeks	Implants	Holes	Tibias
4 (n=6)	1.27* (1.17–1.38)	1.49 (1.44–1.57)	
12 (n=6)	1.33* (1.24–1.45)	1.52 (1.43–1.57)	1.53** (1.46–1.60)
26 (n=7)	1.36* (1.33–1.40)	1.52 (1.46–1.59)	

* Differ significantly from the corresponding hole values and from the tibia values ($p < 0.01$).
** Means for all intervals pooled as stated in Table 2.

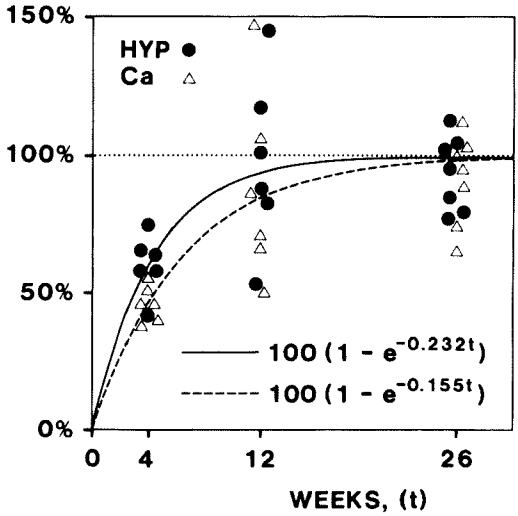

Figure 3. The amounts of hydroxyproline and calcium of tissue within implants have been expressed as percentages of their theoretical maxima given by Equation 4) and 5) (see text) and plotted as scatter diagrams. The growth curves have been drawn as the graphs of equations 6) and 7), with the maxima 11.95 and 246.7 μmol set to 100 per cent. The solid line symbolizes hydroxyproline (HYP), and the dashed line calcium (Ca).

found to be 0.97, indicating a good fit between the data and a straight line. As can be observed in Figure 2, the mineral deposits in the tibias also tended to decrease in the same pattern as those in the implants and holes. To rule out the possibility that the high correlation coefficient between implants and holes is due to a common association with the tibias, i.e. the normal bone metabolism, a partial correlation coefficient between implants and holes was computed. This was found to be 0.96. Thus, it was considered justified to describe the relationship between mineral deposits in implants and holes by a straight line. Model checking as described by Bhattacharyya & Johnson (1977) confirmed the validity of the linear model. The regression coefficients for regression of implants on holes (1.76) and holes on implants (0.54) were significantly different from 1.00 ($p < 0.005$).

Fitting of the hydroxyproline (HYP) and calcium of the ingrown bone to the growth model yielded:

$$4)\quad HYP(t) = 11.95\,(1 - \exp(-0.232\,t))$$
$$5)\quad Ca(t) = 246.7\,(1 - \exp(-0.155\,t))$$

When the upper limits (11.95 and 246.7) are set to 100 per cent, the equations read:

$$6)\quad HYP(t) = 100\,(1 - \exp(-0.232\,t))$$
$$7)\quad Ca(t) = 100\,(1 - \exp(-0.155\,t))$$

The graphs of Equations 6) and 7) are drawn in Figure 3 where the amounts of hydroxyproline and calcium have been expressed as percentages of their respective maximums and plotted as a scatter diagram.

Discussion

The study was undertaken to assess the quality of bone that invades porous fiber Ti-6Al-4V implants. The constituents of the invading bone were assessed by means of chemical methods.

Bone invaded the porous implants rapidly. At 26 weeks the implants had been penetrated by fully mineralized bone to an estimated average depth of 0.45 mm. This means that ingrowth had proceeded to close to two wire thicknesses. Although it is not likely that ingrowth had been evenly distributed over the implant surface, it should confirm an extensive interlock at the bone-implant interface. We admit that the figure 0.45 mm is only theoretical and rests on certain assumptions, but believe it to be a conservative estimate. Andersson et al. (1978) have demonstrated that bone can penetrate this kind of porous material much deeper than 0.45 mm. The specific gravity of bone, 1.64 mg mm^{-3}, agrees well with values given by Albright (1979), when adjusted for water content.

The Ca/P-ratios for the implant-bone were low compared with the bone in the holes and the tibias. Proposal of any causative factors may only be speculative. The larger diameter of the trephine (5.2 mm) compared to the drilled holes (4.7 mm) must have added to the hole samples some old bone with high mineralization and a high Ca/P-ratio. Any eccentric positioning of the trephine would have changed the results in the same direction. The lower ratios observed for the implant bone suggest recently mineralized tissue, although most of them fell within the normal range for mammalian bones (Rasmussen & Bordier 1974). The tibial Ca/P-ratio of 1.53 agrees with previous measurements in feline bones (Balmain et al. 1982).

Factors that govern the ingrowth of bone may be of a nutritive or spatially restrictive nature. Another factor could be lack of the mechanical stress stimulus that is considered to be a prerequisite for bone formation (Klawitter & Hulbert 1971). We calculated that the hydroxyproline and calcium in the ingrown bone tend to 11.95 and 246.7 μmol as t tends to infinity. Moreover, the mineralization will tend to 246.7/11.95 = 20.6, which agrees well with our samples from mature bone. The growth rates of calcium (i.e. the derivative of Ca(t)) at 4, 12 and 26 weeks would be expected to be at least proportional to the mineral deposits (expressed as mol/week) that have been calculated from the isotope data. The expected proportionality is an implication of the accepted rationale for using incorporation of radiotracers as a relative measure of bone formation (Bauer et al. 1961, Heaney 1963). As our design does not discern incorporation due to

exchange from that due to bone formation, a proportionality is the best one can arrive at. If the calculated mean deposits are divided by the relevant derivatives, the quotients are vastly different; 2.7 (4 weeks), 6.8 (12 weeks) and 39.6 (26 weeks). This indicates that as the time intervals increase, there is a shift in the mineral deposit so that an increasing fraction of it participates in exchange mechanisms or is used to replace old bone in a remodelling process. This should be a warning against comparing bone tracer data unless their sources are bone samples of about the same maturity.

The graphs in Figure 3 show that the bone in the implants is deficiently mineralized at the 4 and 12 weeks intervals.

The strict linear relationship between mineral deposits in implants and holes indicates that bone ingrowth and healing of a bone defect are similar processes (Figure 2). The lower deposits for the holes may either be real or may be attributable to the technique used to remove the tissue from the holes, as described above. Healing of experimental lesions like the holes seems to parallel healing of fractures, especially where the bone ends are displaced leaving gaps that have to be bridged (Sevitt 1981).

There are no previous reports on the metabolism of bone invading porous implants. The evaluation has largely been based on histological, mechanical and radiological methods. Provided that the implants were bio-compatible and had adequate pore dimensions, the investigators have repeatedly concluded that the invading bone has a normal maturation to lamellar bone with Haversian systems (Klawitter & Hulbert 1971, Benum et al. 1977, Andersson et al. 1978, Martens et al. 1980, Ronningen et al. 1983). The present study has provided additional evidence that bone within a porous implant is of normal quality and shows normal biological behaviour.

Acknowledgements

The authors thank Dr. med. vet. Leif Schjerven, Mr. Torolf Løvstad and Miss Birgitte Sæthren for their expert technical assistance.

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