Osseointegration of Titanium Ti-6Al-4V Alloy Implants in the Rat Femur: A Time-course SEM Study.

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Biological fixation of uncemented endosseous implants requires the apposition of bone onto implant's surface in a process named osseointegration [1]. The present study examined bone tissue response to Ti-6Al-4V alloy implanted into the rat femurs over a long time range (3 days to 6 months). The progression of implants fixation in the cancellous bone of distal epiphysis and within the medullary cavity of the rat femur was evaluated by SEM, oxytetracycline incorporation and ALP, TRAP and NSE histochemistry.

The implantation and evaluation procedures were performed as described previously [2]. Briefly, Ti-6Al-4V alloy pins were implanted in the distal femurs of 6-month-old Wistar female rats. Animals were euthanized at 3 days, 10 days, 1, 2 and 6 months after implantation. To evaluate mineralization, animals received 30 mg/kg body weight of oxytetracycline, 72 hrs before sacrificed. Undecalcified sections of the femurs with implants were photographed in backscatter mode by scanning electron microscope (SEM). Morphometric analyses were performed using ImagePro software, as described previously [2]. Two parameters of osseointegration: bone volume (BV) and bone-implant contact (BIC) were measured on SEM images of cross-sections from femoral epiphyses and diaphyses. Bone volume was measured within a distance of 0.3 mm from implants' surface.

SEM revealed that after six months successful osseointegration was achieved both in the epiphyseal (Fig. 1A-D) and diaphyseal sites (Fig. 2A-D). Throughout the follow-up period, implant portion located within the cancellous bone of the epiphysis remained in close contact with bone trabeculae that gradually engulfed the implant forming a bone collar continuous with the trabecular network of the epiphysis. In the diaphysis, initially woven bone was first formed within the marrow cavity around the implant; the newly formed trabeculae were resorbed during the later post-implantation period leaving a shell of compact bone around the implant at 6 months (Fig. 2 D). In general it appears that throughout the study period bone formation exceeded bone resorption, as indicated by high osteoblastic (ALP) and mineralization (UV fluorescence) activity.

In conclusion, our study followed up the time course of osseointegration of Ti-6Al-4V alloy implants in the rat femurs over a time ranging from 3 days and up to 6 months.

References

[1] P.I. Branemark et al., *Quintessence* (1985) 11.
[2] G. Sovak et al., *J. Bone Joint Surg.* [Br]; 82-B (2000) 290.



Fig. 1. SEM images of transverse sections of the epiphyses of rat femurs. A,10 days; B, 1 month; C, 2 months and D, 6 months after implantation. TB, trabecular bone.



Fig 2. SEM images of transverse sections of the diaphyses of rat femurs. A,10 days; B, 1 month; C, 2 months and D, 6 months after implantation. BM,; Note that no only bone marrow (BM) is seen in the medullary cavity of the femur at 10 days (A), while trabecular bone appears later on (B-D).