We studied the effects of irradiation on the reintegration of autologous osteoarticular grafts over a period of 24 weeks in a canine model. In 16 foxhounds the medial femoral condyle was resected, irradiated and immediately replanted. In the control group resection and replantation were performed without irradiation. Reintegration was assessed by macroscopic analysis, histology, radiography and gait analysis.

Reintegration was equal at 12 weeks, but significantly inferior in the irradiated group after 24 weeks with delayed bone remodelling. The articular cartilage showed modest degeneration. Conventional radiography and histology showed corresponding changes. Limb function was adequate but the gait was inferior in the treated group.

The rate of survival of patients with primary malignant bone tumours has improved from less than 20% to approximately 80%, mainly as a result of advances in imaging and chemotheraphy. Modern protocols of multidisciplinary treatment with limb-salvage procedures have similar outcomes to those of previous ablative surgery. Techniques of local therapy have become more sophisticated to satisfy the demand for a functional and durable limb. All established reconstructive techniques have well-documented high rates of specific problems and complications. Alternative methods for the reconstruction of the defect require proper evaluation with no change in the protocol of systemic therapy.

Reports on replantation of tumour-bearing bone after external irradiation are encouraging. The tumour is resected with wide margins according to the surgical staging system of the American Musculo-Skeletal Tumour Society. Obvious tumour is cleared before treatment by single-fraction x-ray or electron-beam irradiation. Reconstruction is performed by immediate replantation of the devitalised graft together with stabilisation by osteosynthesis. Sufficient bone stock must be preserved but additional bone grafting, or filling with bone cement, can be performed. The operation can be carried out as a single or as a two-step procedure with intermediate positioning of a spacer depending on the facilities for irradiation which are available. The required dose for safe devitalisation or inactivation of a tumour is not established but large doses of irradiation in the order of megavars, which are considered necessary for sterilisation, will cause damage to the structure of the bone protein and therefore may not be the minimal essential dose. Little systematic work has been undertaken to evaluate the incorporation of irradiated autologous grafts or the function of the affected limbs. All previous experimental canine models have studied replantation of bone segments. The reconstruction of joints is more important clinically.

We have examined the reintegration of extracorporeal irradiated autologous osteoarticular grafts in a canine model and compared the effects of explantation, irradiation and replantation with those of explantation and replantation alone. The null hypothesis was that reintegration of autologous osteoarticular grafts after irradiation with 25 Gy is the same as the reintegration of non-irradiated grafts. Assessment of the process of reintegration of the grafts was by clinical and functional assessment, conventional radiological analysis and histological evaluation of postmortem specimens. The format of this report is adapted to the Consolidated Standards of Reporting Trials (CONSORT).

Materials and Methods

We randomised 32 adult male foxhounds into four groups of eight animals as follows: irradiated (group IA) and control (group IB) with a follow-up of 12 weeks and irradiated (group IIA) and control (group IIB) with a follow-up of 24 weeks.
The animals were randomised to the treated or the control group and to the date of killing by casting lots before the beginning of the study. Three data sheets were generated for each animal and sealed separately in opaque envelopes which were marked with the ID-number of the individual animal. The first data sheet, for the exclusive use of the radiation therapist, contained the assignment for the treatment or control. The second, for the exclusive use of the veterinary surgeon, contained the date of planned killing. The third contained all the information and was held in trust by the generator of the randomisation and handed out to the data analyst only after all information had been collected. Neither the radiation therapist nor the veterinary surgeon participated in the further care of the animals, in the outcome assessments or analysis of data. The assessors were blinded and did not participate in experiments or in the analysis of data. The orthopaedic surgeons did not participate in outcome assessment or analysis of data.

Under general anaesthesia 2.5 cm of the right medial femoral condyle was resected by a fine oscillating saw and a microchisel. The line of osteotomy did not compromise the insertion of the cruciate ligament. The resected specimen was wrapped in saline-soaked gauze for transportation. While the animals were still under anaesthesia, the specimens of the treated groups were irradiated with a single dose of 25 Gy using a 15 MeV linear accelerator. The control groups were not exposed to irradiation. Explantation time was 120 minutes for all groups. Replantation and fixation were achieved by osteosynthesis using AO/ASIF titanium screws. The leg was immobilised in a special splint for the first postoperative day after which the animals were allowed free movement. Prophylactic antibiotics (cefuroxime, 1578 mg) were given intravenously in a single dose before operation and an oral anti-inflammatory agent (diclofenac, 46.5 mg) was given twice a day for two days. After 12 or 24 weeks the animals were killed by an intravenous overdose of pentobarbital.

Reintegration was assessed by a system of established scores using radiological evaluation, gait analysis and macroscopic assessment of the resected specimens post mortem. Radiographs were taken in two planes at 12, 20 and 24 weeks and evaluated for fusion, resorption, the presence of subchondral bone and fracture, fixation, subluxation, graft shortening and narrowing of the joint space according to the ISOLS Radiological Implants Evaluation System. Weekly gait analysis was scored assessing loading on four legs, loading on hind legs, loading at pace and loading at a trot. Both knees were harvested postmortem and the synovium, patella, femoral joint surface and tibial joint surface were examined. The results were quantified according to the score of Ficat, Philippe and Hungerford. The specimens were cut using a precision ribbon-saw in the frontal plane to produce 4 mm slices. Direct-contact radiographs of these slices were taken and the findings were classified according to the healing of the osteotomy line and the structure of the replanted graft.

We used histological evaluation of bone and cartilage as primary outcome measures. The prepared slices were fixed with buffered formalin, dehydrated and embedded in polyester-resin, sectioned at 3 µm and stained with Toluidine Blue and Safranin-O Fast Green iron haematoxylin. The histomorphological findings of bony union, spongiosa and bone marrow were rated according to the score of Heiple, Chase and Herndon. Histological and histochemical analysis of the cartilage was rated according to the structure of Mankin et al for the structure of cartilage, cellularity, Safranin-O staining and integrity of the tidemark. In two animals (one of group IIA and one of group IIB) plastination of the right hind leg was performed according to the technique of von Hagens. All findings were described and classified by two independent assessors. In the case of a discordant judgement a third opinion was taken.

**Statistical analysis.** Since the range of the scores used is different, the results were calculated as a percentage of the maximum score rating. For primary outcome measures and histological evaluation we used the non-parametric Mann-Whitney U test. Two U tests were performed and in order to maintain the error level of α for both tests, a sequential Bonferroni procedure was employed. The calculated unadjusted p values were ordered so that \( p_1 < p_2 \) and both \( p_i \) were compared with \( \alpha/(n-i+1) \). That is, \( p_1 \) was compared with \( \alpha/2 \), and \( p_2 \) with \( \alpha/1 \). As \( \alpha \) had to be 0.05 or less to assign significance to a single test, \( p_1 \) was compared with 0.025, and \( p_2 \) with 0.05. Two-tailed tests were performed. First, we compared the animals which were killed after 24 weeks. If there were significant differences, tests were repeated for the animals killed after 12 weeks. Otherwise no more testing was performed. As hypotheses were a priori ordered for trials lasting for 24 weeks and 12 weeks, an additional adjustment of \( \alpha \) was not necessary. Secondary outcome measures (explorative analysis) were compared by the Mann-Whitney U test. The calculated p values were not adjusted and as a result reported p values <0.05 are not necessarily significant. This should always be borne in mind when reading the explorative part of our results. The values in the tables are given either as the median and the interquartile range (IQR, 25th percentile to 75th percentile) or as the mean ± SD. We used the SPSS for Windows 7.5.1 statistical package (SPSS, Chicago, Illinois) for statistical analysis.

Minor local complications, which are often observed in reconstructive surgery for malignant bone tumours, were not a reason for withdrawal from the trial for a single animal or one of the groups. Severe infection, requirement of additional medication, wound breakdown or premature death were defined as individual stopping rules.

**Results**

The trial was completed in all animals without major complications. There were two minor complications. Breakage of one of the two fixation screws (group IIA) was
noticed on the radiograph at week 12, but revision was not necessary. In another animal (group IA) delayed wound healing occurred ten days after surgery but this resolved after subcutaneous drainage of the haematoma.

**Secondary outcome measures.** According to the ISOLS graft evaluation score more than 75% of the osteotomy line had healed by 12 weeks after surgery in all animals of both control groups. At week 24, union was complete in four animals of control group IIB but was rated as only 75% in seven animals in the experimental group. Graft resorption was less in the control groups compared with the treated groups. Resorption of less than 25% was seen in seven animals in group IIB but only in two in group IIA. One screw breakage was seen (group IIA), without evident separation of the screw fragments. Subluxation, shortening of the graft and narrowing of the joint space were rated normal in all groups. The summarised ISOLS score ratings were less when comparing the treated group with control animals. No significant difference was found between the treated and control group at 12 weeks but at 24 weeks there was a difference when comparing union of the osteotomy line and the structure of the replanted graft using contact radiographs. We performed 576 observations on gait analysis (Fig. 1). In general, the animals did not use the operated leg for one week but thereafter began to bear load and at four weeks were fully weight-bearing on the right hind leg when standing. When walking at a pace or a trot, however, they spared it until week 6. After week 8 the ratings in both control groups were better than in both treated groups. There was no difference at week 12 when comparing treated with control groups, but at week 24 gait analysis in the treated group was slightly worse (Table I).

**Primary outcome measures.** No difference was seen between the treated and control groups at week 12 but at week 24 differences were apparent (Table II). Histological evaluation of the bone according to the score of Heiple et al was similar in groups IA and IB. At week 12, in all but one animal in group IA, the osteotomy had united by...
primary bone healing. In both groups at week 12 only minimum callus formation occurred, mainly on the periosteal side of the metaphysis. Newly-formed trabeculae crossed the former osteotomy site but were less organised in the graft. The spongiosa was filled with fibrous tissue and granulocytes and other haematopoetic cells were seen to invade from the host bone. Endosteal bony resorption by osteoclasts in Howship’s lacunae and repair activity of osteoblasts with broad bands of newly-formed osteoid were observed. Repair activity was seen near the osteotomy site, and to a less extent in the centre of the graft and in the subchondral bone. Since most osteocytes retained their nuclei, the number of empty lacunae was similar in the graft and the host bone. While bony union was equal in group IIA and group IIB at week 24, the morphology of the cancellous bone and regeneration of the bone marrow were superior in the control group. The spongiosa was filled with fatty marrow cells and the diameter of the trabeculae had increased. In the irradiated group active fibroblast-like cells and considerable amounts of haematopoetic cells were present. The trabeculae had become dumpy and thicker, forming a sclerotic and dense spongiosa, with little interstitial space (Fig. 2). Reorganisation continued at 24 weeks with considerable amounts of unresorbed graft. There was a significant difference between treated and control groups in the score of Heiple et al.\textsuperscript{39} at the 24th week (Table II).

The histological and histochemical findings in the cartilage were similar in both groups according to the score of Mankin et al.\textsuperscript{40} at the 12th week (Table II). The thickness of the cartilage was equal in all animals and there were no surface irregularities and differences in cellularity in either group. The chondrocytes were round in the tangential and transitional and more oval in the radial zones with typical quadruplet chondromas; the nuclei were well stained. Cluster formation or cloning was not seen. Clefts to the transitional and radial zone, but not to the tidemark, were slightly more common in the irradiated grafts. Safranin-O staining of the mucopolysaccharides in the ground substance of the cartilage was similar and the integrity of the tidemark was equal in group IA and group IB. At the 24th week the regular thickness of the cartilage was still preserved in both groups, but while the surface was mostly normal in group IIB paleness of the surface and some clefts were seen in group IIA. In two of seven cases diffuse hypercellularity and in one of seven cases the beginning of vascularisation of the tidemark were seen 24 weeks after irradiation. Safranin-O staining was slightly decreased in the irradiation group. The Mankin score was significantly less in group IIA compared with group IIB (Table II).

**Discussion**

The reintegration of extracorporeal irradiated osteoarticular autologous grafts has been evaluated according to a standardised protocol in a canine model. The data obtained complete previous experimental examinations on the

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**Table I.** Details of secondary outcome measures for the treated groups (IA and IIA) and the control groups (IB and IIB) (see text). All measurements were made on eight animals except for contact radiography and the Ficat score which were in seven animals.

<table>
<thead>
<tr>
<th></th>
<th>IA</th>
<th>IB</th>
<th>p value</th>
<th>IIA</th>
<th>IIB</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gait analysis</td>
<td></td>
<td></td>
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<td></td>
<td></td>
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<tr>
<td>Week 12</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>12</td>
<td>93.8 (78.1 to 100.0)</td>
<td>100.0 (100.0 to 100.0)</td>
<td>0.179</td>
<td>87.5 (80.0 to 91.0)</td>
<td>100.0 (90.6 to 100.0)</td>
<td>0.088</td>
</tr>
<tr>
<td>Week 24</td>
<td></td>
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<td></td>
</tr>
<tr>
<td>ISOLS score</td>
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<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Week 12</td>
<td>90.0 (83.0 to 92.0)</td>
<td>94.0 (88.0 to 96.0)</td>
<td>0.091</td>
<td>88.0 (80.0 to 91.0)</td>
<td>92.0 (92.0 to 95.0)</td>
<td>0.014</td>
</tr>
<tr>
<td>Week 20</td>
<td></td>
<td></td>
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<td>Week 24</td>
<td></td>
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<td></td>
</tr>
<tr>
<td>Contact radiography</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>63.0 (53.0 to 75.0)</td>
<td>75.0 (75.0 to 84.0)</td>
<td>0.111</td>
<td>50.0 (38.0 to 63.0)</td>
<td>75.0 (50.0 to 88.0)</td>
<td>0.016</td>
<td></td>
</tr>
<tr>
<td>Ficat score</td>
<td>79.0 (69.0 to 83.0)</td>
<td>83.0 (75.0 to 83.0)</td>
<td>0.510</td>
<td>50.0 (17.0 to 67.0)</td>
<td>67.0 (67.0 to 75.0)</td>
<td>0.009</td>
</tr>
</tbody>
</table>

**Table II.** Details of primary outcome measures for the treated (IA and IIA) and control groups (IB and IIB) (see text). Calculated p values are compared with adjusted p values.*

<table>
<thead>
<tr>
<th></th>
<th>IA (n = 8)</th>
<th>IB (n = 8)</th>
<th>p values</th>
<th>IIA (n &gt; 7)*</th>
<th>IIB (n &gt; 7)*</th>
<th>p values</th>
</tr>
</thead>
<tbody>
<tr>
<td>Heiple score</td>
<td>72.5 (62.5 to 80.0)</td>
<td>90.0 (70.0 to 90.0)</td>
<td>NS (0.258 &gt; 0.025)</td>
<td>60.0 (60.0 to 80.0)</td>
<td>90.0 (80.0 to 100.0)</td>
<td>0.006 &lt; 0.025</td>
</tr>
<tr>
<td>Mankin score</td>
<td>91.0 (88.0 to 94.0)</td>
<td>88.0 (88.0 to 92.0)</td>
<td>NS (0.804 &gt; 0.050)</td>
<td>88.0 (13.0 to 88.0)</td>
<td>100.0 (94.0 to 100.0)</td>
<td>0.022 &lt; 0.05</td>
</tr>
</tbody>
</table>

* measurements at week 24 were based on seven animals
replantation of bone segments after extracorporeal irradiation. Explanation of the observed findings is difficult since the effects of vascular deprivation by the surgical resection and that of irradiation are superimposed. In this model the effects of the surgical procedure are reflected by the control group and different findings are caused exclusively by the irradiation. Thus the efforts of the protocol used achieved as objective an evaluation as possible.

Several aspects of the study must be considered. The dose of irradiation required to achieve sufficient tumoricidal effects could not be selected with certainty since it varies in different primary malignant bone tumours and is not established. Tolerance to irradiation, e.g., in irradiated rabbit mandibles, was noted at 2500 R but not at 5000 R Co. Higher doses are likely to intensify the effects on the metabolism of bone and cartilage rather than to induce a different pathomechanism. Compared with irradiation in situ variations are needed because of the oxygen effect.

The clinical use of extracorporeal irradiation may require doses of 50 to 100 Gy. Variations in chemotherapy, microvascular anastomosis of the graft, immobilisation of the affected limb, additional bone transplantation procedures and other important factors e.g., graft size, presence of tumour, could not be examined. The canine model is advantageous since previous joint re plantation trials have been performed using dogs. Compared with rodents, which have been used in similar investigations, the vascular pattern, secondary bone structure and metabolic activity index of dogs are more comparable with those of man.

Our findings concerning the cartilage and joint surface may not be applied directly to man because of basic differences in the mechanical properties of the articular cartilage.

Since during the first weeks no differences in gait could be detected between treated and control groups, similar recovery from the surgical trauma and similar primary reintegration of the graft can be assumed. Gait analysis in the treated group was subsequently inferior, but differences from the control group were not significant at the 12th week. Further increments in weight bearing in both groups developed equally. At the 24th week the influence of irradiation was associated with inferior function of the affected leg. Adequate function after this procedure has been reported in the clinical application to both dogs and man. Evaluation of reintegration by the ISOLS system is convenient since radiography is still the principal technique used in follow-up studies on bone and joint reconstruction, and it is well known that the osseous portion of grafts will unite rapidly to the host bed. The decrease in the rating in the treated group between 12 and 24 weeks was associated with the radiological finding of porosity next to condensed areas near the centre of the graft, interpreted as graft resorption. These inferior ratings reflect the delay of remodelling of the graft. The contact radiographs enhanced the finding that irradiation did not affect remodelling in the osteotomy line but led to lasting, and even increasing, resorption processes within the grafts during follow-up.

Histological studies showed that a remodelling process takes place in transplanted grafts. The bone will be resorbed causing initial porosity as early as two months after surgery, reaching a peak at six months. Long-lasting remodel ling will follow. Union of the osteotomy was completed after 12 weeks by primary bone healing in the control groups. The histological evaluation indicated that irradiation did not influence the process of bone union and graft reorganisation around the osteotomy at the 12th or at the 24th week. This agrees with findings after application of this technique in man. Marrow stroma and endosteal lining cells, which are the main contributors to osteogenesis within grafts, were equally distributed in treated and control groups at the 12th week. In the control group at the 24th week the morphology was almost normal with only a moderate increase in the diameter of the trabeculae. Reorganisation was different in the central parts of the irradiated grafts. Compared with the control group, the trabeculae had become dumpy and thicker, and active fibroblast-like cells and haematopoetic cells were present. Bone remodelling by osteoclasts and osteocytes was still very active. Osteocytes, as postmitotic cells, are considered to be relatively radioresistant because of their slow turnover but it has been shown that their viability is reduced by irradiation. RNA synthesis is rapidly reduced but a decrease of viable osteocytes occurs slowly as a delayed process. Direct cell damage, inflammatory reactions and induction of reproductive cell death are responsible for the death of osteocytes. Our finding of late increased activity of osteocytes within the irradiated graft resulting in sclerotic bone may well be substantiated by the speculation of Takahashi et al of a possible release of local growth factors which stimulate the activity of osteoclasts during the acute inflammatory phase after irradiation. In our model reorganisation of the grafts was altered and protracted but not stopped by irradiation with 25 Gy. Whether the central parts of the irradiated grafts will be remodelled completely could not be evaluated due to the limited follow-up of our study.

Our findings on the cartilage were similar at the 12th week, but inferior at the 24th when comparing the treated with the control groups. In non-irradiated autograft transplantation articular cartilage was reported to maintain its shape for a long time but with moderate degenerative changes. Thus our results showing almost undisturbed structure and equal staining of the mucopolysaccharides in the control groups are reliable. At the 24th week after irradiation the thickness of the articular cartilage was preserved but moderate degenerative changes, with superficial clefts and hypercellularity, were observed. Inferior Ficat and Mankin scores in irradiated grafts compared with the control group at the 24th week in our investigation seem to differ from recent reports on the high tolerance of articular cartilage to irradiation of 50 Gy, which shows no obvious degenerative changes, normal architecture of the collagen
and active RNA synthesis.55 These results were achieved in rabbits but degenerative changes comparable to ours have been described in dogs at 10 and 40 Gy by others.62 Late regenerative processes are possible in primarily dead cartilage (allografts).64

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No benefits in any form have been received or will be received from a commercial party related directly or indirectly to the subject of this article.

References

46. Wannenmacher M. Experimentelle und klinische Untersuchungen zur Wirkung ionisierender Strahlen auf die Gewebe im Bereich der Mundhöhle. München, etc: Verlag, 1976.


