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Diaphyseal reconstruction by autoclaved bone

Reimplantation experiments in rabbits

In 8 adult rabbits, reconstruction of large humeral defects by reimplantation of resected, autoclaved bone supplemented with allogeneic bone matrix was investigated with respect to incorporation (radiography, histology), bone metabolic activity (scintigraphy, autoradiography), and strength (torsional test). Radiography showed that seven out of eight implants were incorporated at 3 months. Scintigraphy and autoradiography disclosed bone metabolic activity in the reconstructions still 8 months postoperatively. Histological investigations evidenced abundant new viable bone that partially had replaced the implants at 8 months. The torsional test disclosed that the strength of the reconstructions was 84 per cent of normal 8 months after reimplantation. Our experiment supports the concept of reimplantation of autoclaved tumorous bone supplemented with allogeneic bone matrix in reconstruction after local resection.

Andris Kreicbergs
Peter Köhler

Department of Orthopedics,
Karolinska Hospital,
S-10401 Stockholm, Sweden

Reconstruction of large skeletal defects following local resection in the treatment of malignant bone tumors is a surgical problem. Resected tumorous bone reimplanted after autoclaving has been reported to offer a simple means for both tumor devitalization and reconstruction (Johnston et al. 1983, Smith 1975, Thompson & Staggall 1956).

In previous studies in rabbit, we used autoclaved reimplants and allogeneic transplants for reconstruction of large ulnar defects (Köhler et al. 1987) and found poor new bone formation and a high rate of nonunion. Therefore, allogeneic bone matrix, known to be a potent bone inductive substance (Urist et al. 1967, Urist et al. 1983) was supplemented to similar reconstructions (Köhler et al. 1987, Köhler & Kreicbergs 1987). The reconstructions then exhibited abundant new bone formation and the implants incorporated consistently within 16 weeks.

We can now report that reconstructions in the rabbit by reimplanted, autoclaved bone supplemented with allogeneic bone matrix retain bone metabolic activity and are eventually capable of meeting normal mechanical demands.

Materials and methods

The middle third of the humerus including the periosteum was resected unilaterally (left and right, randomly chosen) with oblique osteotomies, autoclaved at 121° C for 20 min and subsequently reimplanted in 11 adult rabbits under anesthesia. The animals were anesthetized by i.m. injection containing a mixture of fluanizonium 10 mg and fentanyl citrate 0.315 mg/ml (Hypnorm, Leo), given in a dose of 0.3 ml/kg body weight combined with diazepam 1.5 mg/kg (Valium, Roche). Two intramedullary pins of different lengths were used for fixation of each implant (Figure 1). The shorter pin was inserted in the wider proximal osteotomy to prevent tilting of the implant. Pieces of allogeneic bone matrix, weighing approximately 200 mg were placed along the entire length of each reconstruction. Bone matrix was prepared according to Urist from cortical diaphyseal bone of fresh rabbit cadavers as previously described (Köhler et al. 1987). The contralateral humerus was left intact to serve as a control. The animals, kept in cages, were allowed full weight bearing postoperatively. After 3 months the animals were let out of the cages twice a week to move around freely for 1 hour.

At 6 months the longer fixation pin was removed and 2 months later, i.e., 8 months postoperatively, the animals were killed by an i.v. overdose of Mebumal, ACO (to mg/ml) and both humeri of each animal were collected.

The reconstructions were studied in vivo under anesthesia with conventional radiography 6 weeks, 3, 6, and 8 months postoperatively, and with scintigraphy 6 and 8 months postoperatively in a maxicamera 400 T (General Electric) connected to a Gamma-11 image-processing system (Digital Equip-

Figure 1. The incorporation process of reimplanted autoclaved bone in humeral reconstruction supplemented with allogeneic bone matrix. A Immediately, B 6 weeks, and C 3 months postoperatively.

ment) 2 hours after i.v. administration of 50 MBq ^{99m}Tc-MDP.

Postmortem investigation of the reconstructions included high-resolution radiography in a Faxitrone (Hewlett Packard) at 35 kV/144 mAs using Industrex M film (Kodak), autoradiography (i.v. 30 MBq ^{45}Ca 2 days before death) in 35 µm-thick tissue sections attached to Structurix D7 (Agfa Gevaert) film for 7 days (Rohlin et al. 1977), and histologic analysis in Weigarts hematoxylin and in van Gieson-stained sections.

The structural properties of the reconstructed and the intact contralateral humeri were examined in a computerized torsional test machine (Johnsson & Strömberg 1985). Both ends of each specimen were firmly fixed and the distal end was twisted inwards at the constant speed of 6 degrees/sec until fracture. Simultaneously, the torque twist curve was recorded by an x-y plotter. From each curve, torsional strength (maximum torque capacity) and stiffness (linear torque twist relationship) were derived. For each animal the ratio between the reconstructed and the contralateral humerus was calculated with respect to strength and stiffness.

In 3 animals, the longer pin dislocated upwards within 6 weeks postoperatively. In one of them a pin penetration caused infection and the rabbit was killed. In the other 2 rabbits, the pin was repositioned surgically; 1 of these developed nonunion. One animal had a peroperative injury of the radial nerve and was discarded. Another rabbit died of intestinal obstruction 2 months postoperatively. Seven out of 8 animals completing the entire 8-month experiment were found to have healed reconstructions. The following observations were based on these 7 animals.

Results

Radiography (Figure 1) showed abundant new bone along the entire length of the reconstructions already at 6 weeks. Incorporation was observed at 3 months. During the rest of the study, the reconstructions remained radiographically essentially unchanged, apart form signs of slow resorption of the implants.

Scintigraphically, there was clearly an increased ^{99m}Tc-MDP uptake in the reconstructed humeri at both 6 and 8 months (Figure 2).

High-resolution radiography of the reconstructed humeral specimens showed complete incorporation of the implants (Figure 3). Autoradiography (Figure 4) disclosed deposition of ^{45}Ca within, as well as around, the implant, indicating that active bone formation still took place in the reconstructions 8 months postoperatively.

Histologic analysis showed abundant viable new bone along the entire length of the reconstructions. New bone with viable marrow tis-

Figure 2. Scintigraphy showing increased ^{99m}Tc-MDP uptake 8 months postoperatively in a humerus reconstructed by reimplantation of autoclaved bone supplemented with allogeneic bone matrix.

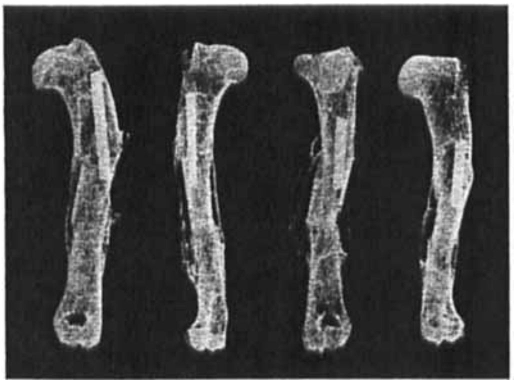


Figure 3. High-resolution radiography of four different humeral specimens at 8 months postoperatively showing complete incorporation of autoclaved reimplants supplemented with allogeneic bone matrix.

Table 1. Structural properties of the humeral reconstructions.

	Ratio reconstructed/intact humerus	
Animal no	*Strength*	*Stiffness*
1	*0.91*	*1.81*
2	*0.93*	*1.39*
3	*0.85*	*1.36*
4	*0.71*	*1.17*
5	*0.78*	*1.40*
6	*0.76*	*1.22*
7	*0.96*	*1.48*
8[a]	*0.22*	*0.38*
Mean (SD)	*0.84 (0,10)*	*1.40 (0.21)*

[a] *Non-union: values excluded from calculation.*

sue was found to have replaced implant bone to a large extent. The remainders of implant bone, in many areas hardly discernible, appeared nonviable. Newly formed bone was in intimate contact with implant bone both in the osteotomy area and along the implant (Figure 5). There were no signs of inflammatory reaction.

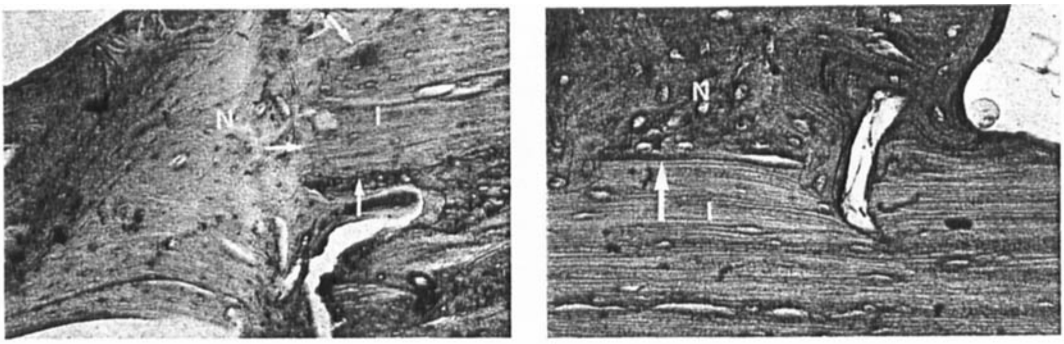
The torsional test of the reconstructed humeri, as well as the contralateral intact humeri, consistently caused a spiral fracture in the mid-diaphyseal segment. The strength of the reconstructed humeri was 84 per cent of the contralateral humeri. Stiffness, on the other hand, was increased by 40 per cent (Table 1).

Discussion

Our experiment shows that permanent, stable reconstruction of large diaphyseal defects can be attained by reimplantation of resected, autoclaved bone supplemented with allogeneic bone matrix.

Previous experimental studies have shown that large skeletal defects may heal after supplementation with bone matrix, known to be a potent bone inductive substance (Gupta et al. 1982, Einhorn et al. 1984, Oikarinen et al. 1979, Takagi et al. 1982). However, the substance alone does not provide immediate mechanical stability. In the reconstruction of weight-bearing bone, the inductive substance has to be combined with a stabilizing system until osseous bridging of sufficient strength is reached. Autoclaved reimplanted bone, apart from being mechanically supportive (Köhler et al. 1986), represents a sterile, easily accessible graft of exact size and form. Combined with allogeneic bone matrix, it offers a reconstruction with high osteogenic capability. After implant incorporation the reconstruction seems to remain viable even in the long run. The application of analogous principles, i.e. antigen-extracted, autodigested, allogeneic bone (Urist et al. 1975, 1981) or frozen allografts combined with bone matrix would probably give similar

Figure 4. Autoradiography of a humeral specimen at 8 months showing ^{45}Ca deposition in autoclaved reimplant (between arrows) and in enveloping new bone.



A B

Figure 5. Histologic section of humeral reconstruction by autoclaved reimplant supplemented with allogeneic bone matrix 8 months postoperatively. Junction between newly formed bone (N) and implant bone (I) in A, osteotomy area (x160) and B, along cortex of implant (x300), is denoted by arrows.

good results. However, by its simplicity, our method appears preferable.

Whether new bone enveloping the autoclaved reimplants mainly was due to bone induction by allogeneic bone matrix or bone conduction could not be determined. Previous studies on ulnar reconstructions (Köhler et al. 1987, Köhler & Kreicbergs 1987) strongly suggest that new bone around the present humeral predominantly originated from bone induction by allogeneic bone matrix. Although autoclaved bone does not induce new bone formation (Köhler 1986, p. 22), it does not represent an inert spacer, such as a metal implant, since it may be replaced by new bone.

It remains undetermined to what extent strength of weight-bearing diaphyseal bone may be reduced and still remain capable of meeting normal mechanical demands. It is, however, reasonable to assume that four fifths of normal strength is quite sufficient (Låftman et al. 1980). To our knowledge, no other mode of reconstructing diaphyseal defects in weight-bearing bone has been shown to provide higher mechanical strength. Weiland et al. (1984) using vascularized autografts for femoral defects in dogs reported an average reconstruction strength of 19 per cent compared with intact femur. Reconstruction of similar defects in the rat utilizing bone matrix yielded 37 per cent of normal strength (Einhorn et al. 1984). In these two studies the low regain in strength may be partly attributed to stress protection of the fixation used (Alho et al. 1982, Strömberg 1975). Provided a skeletal reconstruction permits removal of fixation, as in our model, conditions for strength recovery improve. Further, our reconstructions were not even from the beginning entirely dependent on the internal fixation, because the autoclaved reimplant itself contributed to stability.

Noteworthy, the reconstructed humeri showed an increase in stiffness, which may be explained by differences in cross sectional diameter and length of the compared humeral specimens. The temporary rigid internal fixation may also have contributed to this increase (Mølster) et al. 1982). However, the physiological significance of our finding is probably negligible. In this context, it should be pointed out that the shorter intramedullary pin, which was left in the specimens, hardly can be expected to influence the results of the torsional test, although the pin, theoretically, might contribute to increased bending strength.

We conclude that a combination of autoclaved bone and a bone inductive substance may permit reconstruction of the skeleton so that the bone tissue will develop normal metabolic and mechanical properties.

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