Acta Orthop Scand 1990; 61(6): 546–548

Lipid extraction enhances bank bone incorporation

An experiment in rabbits

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We implanted frozen allogeneic cancellous bone in rabbit skeletal defects and compared the bone-forming response with that from similar implants that had also been extracted with chloroform/methanol. The donor bone was harvested from a previously implanted titanium chamber that is spontaneously filled with reproducible amounts of cancellous bone. It was processed as frozen bank bone, then transferred to an identical, but empty, chamber in another rabbit. Extraction of lipids before implantation increased the ingrowth of new bone into the transferred bone, as measured by ^{45}Ca and ^{99m}Tc-MDP activity. A simple treatment with fat solvents may reduce some of the drawbacks of ordinary bank bone.

Conventional frozen bank bone is associated with the risk of viral contamination. The sometimes tedious preparation of, e.g., a femoral head before implantation may increase the operating time. Further, with frozen cancellous bank bone, most of the implant consists of dead marrow cells, which must be eliminated by the host. Although frozen bank bone functions well for some applications, we hypothesized that a reduction of its necrotic soft-tissue content by lipid extraction may facilitate incorporation.

Material and methods

General disposition

We used pairs of titanium chambers implanted in rabbit tibias. Bone was formed inside these chambers and harvested as 1 x 1 x 5-mm cancellous bone rods (Tjellström et al. 1978). These bone rods were implanted as grafts in pairs of chambers in other rabbits. Before grafting, the cancellous bone rods were frozen, and one in each pair was also defatted.

The bone harvest chamber

The bone harvest chamber (Albrektsson et al. 1984) is a 7-mm-high and 6-mm-wide titanium cylinder, which is threaded so that it can be screwed into bone. It is closed by a pistonlike core, and the entire device is pierced by a 1-mm-diameter transverse canal for bone ingrowth. When the core is removed, the tissue inside the bone ingrowth canal is uncovered, and can be harvested without disturbing the surrounding bone. The chamber can be used for repeated harvesting (Figure 1).

Procedure

Seven, lop-eared, 4.8–5.4-kg, adult (> 9 months) rabbits of both sexes were used. The chambers were inserted bilaterally in the proximal tibias just anterior to the insertion of the medial collateral ligament. The bone ingrowth canals were placed at the level of the cortical bone. The chambers were covered with fascia, and the skin was sutured intracutaneously. After 5–6 weeks, the chambers were

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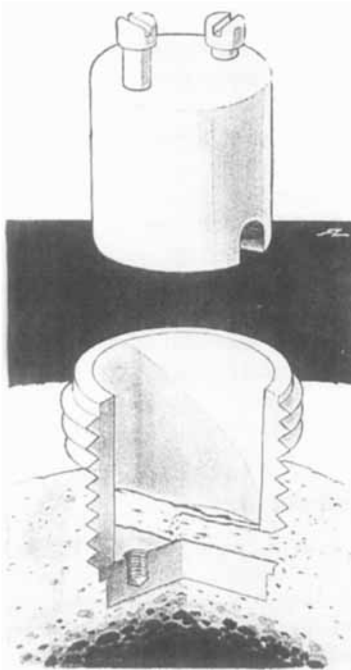

Figure 1. A cancellous bone rod was produced in the Bone Harvest Chamber. The bone rod was frozen, thawed, and implanted in another chamber with or without previous lipid extraction.

Table 1. ^{45}Ca, ^{99m}Tc-MDP and calcium content in cancellous bone grafts from 10 pairs of implants in rabbits. Ratios defatted/non-defatted

	n	Range	Median	*P*
Grafts 3 weeks after implantation				
^{45}Ca	10	1.0–3.4	1.7	0.02
^{99m}Tc-MDP	10	1.0–2.0	1.4	0.02
calcium	10	0.7–1.8	1.3	NS
Grafts when harvested from donor				
^{99m}Tc-MDP	9[a]	0.6–1.3	1.0	NS
Bone removed to make place for graft				
^{99m}Tc-MDP	10	0.7–1.3	1.0	NS

[a]One sample missing.

harvested. The canals contained bone that was not further used. Then, the chambers were ready for use. In the first step, the chambers were closed; and after 5 weeks, they were harvested for cancellous bone rods, which were used as grafts. In the second step, the grafts were implanted in the emptied bone ingrowth canals of the recipient animals' chambers, which were harvested 3 weeks later. Five of the rabbits were used as recipients. At most, each rabbit received three pairs of implants, however, never twice from the same donor.

Graft preparation

The harvested cancellous bone rods were kept sterile at –70 °C. The day before implantation, one in each pair was thawed and deposited in a test tube with 1:1 chloroform-methanol overnight. Before grafting, they were rinsed three times with methanol, three times with water, and finally with saline. The non-defatted graft was then thawed in saline. At implantation, the right and left sides alternately received defatted grafts.

Evaluation

The rabbits received an intravenous injection of 45 MBq ^{99m}Tc-MDP 3 h before each harvest. The activity of ^{99m}Tc-MDP was measured in a well counter immediately after harvest. An intravenous injection of ^{45}Ca (460 kBq/kg body weight) was administered 24 h before the grafts were harvested. The grafts were then ashed in a muffle furnace at 800 °C for 24 h. The ash was dissolved in 6 M HCl, dried in a vacuum centrifuge, and redissolved in 100 mL citrate buffer pH 2.98. Of this, 50 mL was measured in the clinical routine laboratory for serum Ca analysis (DACOS), and 40 mL was mixed with 13 mL of a scintillation cocktail (Opti Phase "HiSafe" II, LKB Wallac) and measured in a liquid scintillation counter (1215 Rackbeta, LKB Wallac).

We used a two-tailed sign test, and assumed that the first and second experiments in each animal were independent (we observed no tendency towards dependence).

Result

The ^{45}Ca and ^{99m}Tc-MDP activities were both higher in the defatted implants in nine out of 10 pairs at the final harvest. The median increase was 70 and 40 percent, respectively (Table 1). The ratio defatted:nondefatted for ^{45}Ca and ^{99m}Tc-MDP activities correlated well ($r = 0.83$; $P < 0.003$). The total calcium content at the final harvest was not increased.

There were no side differences of ^{99m}Tc-MDP activity in the implants when they were harvested from the donors. And there were no side differences of ^{99m}Tc-MDP activity of the bone that was removed to make place for the implanted bone. Finally, there were no left-right side differences.

The median ^{99m}Tc-MDP activity at the final harvest in defatted specimens was 21 (14–37) cpm x 10^3 and in nondefatted specimens 14 (9–34). This magnitude is similar to that of a previous series of nongrafted, 2-week specimens (Aspenberg et al. 1988c). In that series, this magnitude of ^{99m}Tc-MDP activity was associated with high bone-forming activity within most parts of the specimens, as confirmed by histologic studies.

Discussion

Studies of bone grafting in small animals is marred by the scarcity of cancellous bone, and cancellous bone grafts may be difficult to standardize because of local variations at the harvesting site. With our model, the bone to be grafted was formed with a predetermined shape under standardized conditions.

Little is known about the biologic effects of processing bank bone, for example, by lyophilization (Burckhardt 1983). The effect of lipid extraction on our bone grafts may be due to a reduction of both specific immune reactions and general inflammation, which disturb bone formation (Aspenberg et al. 1988b). In the bone ingrowth canal of our titanium chamber, the inflammation caused by minimal amounts of demineralized bone matrix is enough to reduce bone formation (Aspenberg et al. 1988a). Also, the time needed by the recipient to remove all the débris from the bank bone may cause a later onset of bone formation. Perhaps removal of cell-surface antigens by destruction of the cell membranes plays a role.

The common use of sterile, deep-frozen femoral heads for total hip revisions requires tedious graft preparation in the operating room, with associated infection risks and delay. Therefore, we now use preshaped bank bone, of which the soft tissues have been removed with fat solvents and high-pressure irrigation. According to our present findings, this treatment of bank bone may also lead to a more rapid bony incorporation of the graft.

Acknowledgement

We would like to thank Ms. Gerda Seidel and Ms. Stina Nordström for technical assistance. Grants were received from the Swedish Medical Research Council (project 17X–2031), the Medical Faculty of Lund, the Swedish Medical Society, and the Alfred Österlund Foundation.

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