The Repeated Sampling Bone Chamber: A New Permanent Titanium Implant to Study Bone Grafts in the Goat

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Abstract | We developed a repeated sampling bone chamber (RSBC) and tested its suitability for studying various aspects of the bone allograft incorporation process under reproducible non-load-bearing experimental conditions in a large vertebrate. Our chamber is made of commercially pure titanium and is designed to allow bone or tissue ingrowth into a removable hollow inner core. Three chambers per animal were randomly implanted in the tibias of 10 goats and were harvested every 8 weeks. In experiment 1, two chambers were filled with a fresh-frozen structural allograft or a chip allograft, and one was left empty. In experiment 2, all chambers were left empty to measure intra- and interanimal variation. The results were evaluated by histomorphometry. Clinical results of four growth factor experiments also are presented.

Using this model, we conducted 60 harvest operations (median, 4/animal; range, 2 to 8). In experiment 1, more soft tissue ingrowth and osteoclasts were measured in the chambers with allograft (P < 0.005 and P < 0.03 respectively). Bone ingrowth was scant, with no significant differences between chip graft, structural graft, and empty control chamber. Thus, the bone graft did not show any osteoinductive or osteoconductive properties. Experiment 2 indicated consistent tissue ingrowth, with greater interanimal variation than variations among the chambers in any goat. Our method forms a means of studying gradual tissue and bone ingrowth into bone grafts. The inherent low amount of bone ingrowth makes this model suitable for studying bone-inductive substances. Repeated sampling in the same animals lowered the intersample variability and reduced the number of animals that were required.

The amount of bone graft material used in bone reconstructive surgery is rapidly increasing. In the future, the quantities of bone auto- and allograft available may not be sufficient to meet the demands. Furthermore, use of bone grafts involves the risk of disease transmission and requires expensive testing for infective agents. Development of materials to replace bone grafts and the isolation of bone growth-stimulating factors are therefore important.

The graft incorporation and bone healing capacities of various materials and factors have been tested in vivo models (1, 2). These experiments were conducted in small laboratory animals; because the rate and potential for bone repair appears to be inversely related to the size and age of the animal (3), these studies are remote from the human situation. Also, the amount of material that can be tested in these models is limited (1, 2). Other in vivo studies on larger animals were not standardized with respect to the mechanical loading condition (4), which is an important factor in the bone healing process. Therefore, the pure biological influences of the implanted materials were obscured.

We adapted the bone chamber model used in rodents (2) for use in a larger vertebrate to investigate the in vivo influences of bone processing and growth factors on tissue ingrowth and bone ingrowth, implant resorption, and incorporation under reproducible, non-load-bearing conditions. We present the properties of the bone chamber on the basis of results of two experiments.

Materials and Methods

Implant: The repeated sampling bone chamber (RSBC) is made of commercially pure titanium. It consists of a cylindrical outer housing and a removable hollow cylindrical inner core that holds the experimental material (Figure 1). The outer housing is 6.7 mm in diameter and 10 mm high. It is closed at the inner end and is permanently fixed to the proximal metaphyseal portion of the tibia, using two flanges and two cortical screws (2.7 x 12 mm). The hollow cylindrical inner core (diameter, 3 mm; height, 7 mm) can be screwed into the outer housing. The outer housing and the hollow cylindrical inner core have two round ingrowth holes (diameter, 1.5 mm) that are in direct contact with the tibial cortex. The closed inner end of the outer housing ensures undisruptible osseointegration of the chamber, irrespective of harvesting operations. When the chamber is assembled, a peg prevents rotation of the inner cylinder in the outer housing and ensures a stable conduit for tissue and bone ingrowth. Because the tissue in the inner chamber is connected to the host only via the ingrowth holes,

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Figure 1. The repeated sampling bone chamber (RSBC). O = Outer housing, fixed to the proximal medial portion of the tibia by use of two cortical screws. I = Inner core, consisting of a cylinder in halves that can be screwed into the outer housing. P = Security peg, which prevents rotation of the inner core in the outer housing. C = Mushroom-shaped screw cap. After osseointegration of the implant, the bone can grow into the chamber through the two ingrowth holes.

tissue connection is easily disrupted during the sampling procedure. The chamber is closed with a screw cap.

Surgery: Ten mature goats (Capra hircus sana), obtained from our local "Central Animal Laboratory Farm," each received three chambers on the medial aspect of the proximal portion of the tibia, divided between the left and right limbs. The limb that received two chambers was chosen at random. Anesthesia was induced with pentobarbital (Narcovet, 60 mg/ml; Apharmo Med BV, Arnhem, The Netherlands) at a dosage of 0.5 ml/kg of body weight. Goats were intubated, and anesthesia was maintained with halothane and oxygen, using a semiclosed ventilation system. The hair over the lower part of the limbs was shaved, then the skin was washed, painted with an iodine-containing solution, and covered with sterile cloths.

A curved incision was made in the skin and fascia over the medial aspect of the proximal portion of the tibia. A hole was drilled through the medial aspect of the cortex, at approximately 3 cm from the joint cleft, with a 6.7-mm drill with a diamond tip. The drill was connected to a saline solution irrigation system (Surgical Diamond Instruments Scientific Developments, München, Germany) to avoid heat-induced necrosis of the peri-implant bone. In addition, care was taken not to violate the marrow tissue.

After the holes for the cortical screws were drilled, the outer housing was fixed to the tibia with small cortical screws. The chambers were placed so that the ingrowth holes were parallel to the longitudinal axis of the bone. The inner core was screwed in, the peg was placed, and the chamber was closed with the screw cap. In the tibia that received two chambers, these were placed 10 mm apart. During the first implantation period, the chambers were left empty. At each subsequent harvest operation, the inner cylinder was unscrewed; this disrupted any ingrowing tissue and protruding bone through the ingrowth openings. The halves of the inner cylinder were split apart, and the specimen was removed easily without damaging the tissue. New bone graft material could then be placed in the chamber. The fascia and skin were sutured separately after each repeat operation, but special wound dressings were not used in the early postoperative period. During the harvest operations, the animals were anesthetized in similar manner as that used in the chamber implantation operation, but antibiotics were not given after surgery. After the RSBC implantation procedure, the goats received ampicillin (Albupen LA, 100 mg/ml; Mycofarm, De Bilt, The Netherlands), 7.5 ml/d given s.c. for 5 days. After all surgical procedures, the goats were allowed unrestricted movement in their cages and ad libitum access to water and food.

Experimental design: At the initial implantation procedure, the chambers were left empty. After an 8-week postoperative healing period, the three chambers were harvested before the experiments were started. These so-called "priming" specimens were not used for histomorphometry, but served only as controls to ensure that the chamber seating allowed tissue and bone ingrowth. All subsequent harvest operations were performed at 8-week intervals.

In experiment 1, fresh-frozen allografts from a nonrelated donor goat were placed in two of the RSBCs of each goat. The first chamber was filled with impacted chips, the second with a structural trabecular cylinder, and the third chamber was left empty. For all 10 goats, the chambers were used alternately, receiving graft material or remaining empty, so that all localizations were of similar numbers.

In experiment 2, all three chambers were left empty. The specimens were used to investigate intra- and interanimal variability in tissue and bone ingrowth. To substantiate the repeated harvest potency of this model, another four harvest operations per goat were conducted on the group of 10 goats. In these four experiments, the chambers were filled with bone allograft material, with or without one or several locally applied growth factors.

Graft preparation: The allografts were taken under sterile conditions from a donor goat. The comparative homogeneous cancellous bone of the sternum was cut into small chips or drilled from the sternal bone as cancellous cylinders (size, 3 x 7 mm). The graft material was cultured, packed in sterile bags, and stored at -70°C. Before implantation, the grafts were thawed in saline.

Histologic examination: All specimens were used to investigate intra- and interanimal variability in tissue and bone ingrowth. To substantiate the repeated harvest potency of this model, another four harvest operations per goat were conducted on the group of 10 goats. In these four experiments, the chambers were filled with bone allograft material, with or without one or several locally applied growth factors.
sections. Sections were processed in routine manner for non-
decalcified histologic examination (hematoxylin and eosin, according to Mayer and Goldner-Masson trichrome) and 
enzyme histochemistry (alkaline phosphatase [AP] and tarr-
trate-resistant acid phosphatase [TRAP]). Histomor-
phometry was performed with a microscope connected to a 
computerized video digital table system (Videoplan; Kon-
tron Bildanalyse GMBH, Munich, Germany). At magnifica-
tion of 25x, we measured the area of tissue ingrowth (ATI in 
square millimeters), the area of bone ingrowth (ABI in square 
millimeters), and the width of the specimens (WS in milli-
meters). Relative ingrowth variables were calculated for the 
tissue ingrowth distance (ATI/WS) and the bone ingrowth 
distance (ABI/WS). These variables represent the mean lon-
gitudinal ingrowth distance (in millimeters) into the inner 
chamber. Osteoclasts were counted, using a magnification 
of 100x. The reproducibility of the tissue measurements and 
the cell measurements were 2.5 and 5%, respectively.

Statistical evaluation: Results of experiment 1 were 
tested with two-way ANOVA for the factors goat and type of 
implant in the chamber. A simultaneous test to prove pair 
differences between the bone chamber contents was con-
ducted according to the Bonferroni principle. Spearman's 
correlations were calculated for the coherence between tis-
ue ingrowth and number of osteoclasts. Experiment 2 was 
tested with three-way ANOVA for the factors goat, RSBC 
localization, and cortex thickness at the RSBC localization.

Results

Clinical evaluation: After the priming operation, we 
performed 60 operations, comprising three harvests per 
operation, which yielded a total of 180 specimens. We con-
ducted four repeat operations per animal (median), with a 
range of two to eight harvest operations per animal. In 
experiment 1, the specimens of one goat were lost because of 
infection in one of the RSBCs. Six goats had to be replaced 
by new ones during the 60 harvest operations, all because 
of infection in one of the RSBCs. Overall, 1 goat in 10 was 
lost at every harvest operation. Six goats maintained their 
RSBC successfully during all harvest operations. Gener-
ally, all goats tolerated the bone chambers well, with good 
fixation of the implants over a period of 6 to 12 months 
without skin ulceration or wound healing problems. Radi-
oigraphy indicated cortical thickening around the implants 
after 8 weeks, which resulted in evident covering of the 
ingrowth holes by cortical bone.

Harvest evaluation: After the priming period (8 weeks), 
tissue ingrowth had occurred in all the chambers. How-
ever, tissue ingrowth was confined to bridging the empty 
spac between the two ingrowth holes, with no tendency to 
fill the chamber further. The amount of bone in the speci-
mens varied between an absence of bone tissue and the 
presence of a nearly uninterrupted bone layer between the 
two ingrowth holes.

In experiments 1 and 2, the specimens from two goats 
were reserved for future immunohistochemical evaluation 
and therefore could not be processed for histologic examina-
tion in routine manner. In experiment 1, one goat died from 
an infection. Therefore, the specimens from seven goats were 
available for histologic and histomorphometric analyses.

The specimens from experiment 1 had consistent soft tis-
ue ingrowth and variable bone ingrowth. The empty con-	rol chambers contained fibrous tissue with macrophages, and 
sometimes woven bone with a few osteoclasts. In the 
chambers containing graft material, more macrophages 
were present, whereas on the surfaces of the dead bone graft, 
abundant TRAP-positive osteoclasts were found. The in-
ingrowing bone appeared to have been formed by membra-
nous ossification (Figure 2); cartilage was not detected. The 
factor RSBC contents appeared to be significant for the 
tissue ingrowth distance (P < 0.005) and the number of osteo-
clasts (P < 0.03). For bone ingrowth distance, the factor goat 
was significant (P < 0.01), but not the RSBC contents. The 
chambers with the grafts had more tissue ingrowth than 
did the empty controls (3.2 ± 0.5 mm, P < 0.05 and 4.4 ± 0.5 
mm, P < 0.01 respectively) (Table 1). Bone ingrowth was 
scant, with no significant differences between the chip graft, 
the structural graft, and the empty control chamber (0.1 ± 
0.2 mm, 0.5 ± 0.2 mm, and 0.6 ± 0.2 mm respectively).

In experiment 2, none of the experimental factors (goat, 
chamber position, or cortical thickness at the bone cham-
ber localization) were significant. However, the interanimal 
variation was greater than the variation among the cham-
bers in any one goat. The amount of tissue ingrowth and 
the number of osteoclasts varied less than the amount of 
bone ingrowth. The coefficient of variation for tissue in-
growth, number of osteoclasts, and bone ingrowth were 0.3, 
0.8, and 3 respectively.

### Table 1. Mean ± SD histomorphometric results of the bone chamber specimens

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<tr>
<th></th>
<th>CHIP</th>
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<th>STRU</th>
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<th>A</th>
<th>B</th>
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<td>0.5±</td>
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<td>0.6±</td>
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</table>

TID = tissue ingrowth distance in millimeters; BID = bone ingrowth distance in millimeters; OCLAST = number of osteoclasts
CHIP = impacted chip allograft; CONT = control, empty chamber; STRU = structural allograft; BC <2 = bone chamber located in cortex thinner than 2 mm; BC >2 = bone chamber located in cortex thicker than 2 mm
A = proximal chamber of two; B = distal chamber of two; C = single chamber

$$^p < 0.01; ^2 < 0.02; ^3 < 0.05$$
Figure 2. Photomicrographs of sections of a fresh-frozen allograft implant with osteoclastic bone resorption after 8 weeks (A) and tissue from a control RSCB after 8 weeks (B), and detail of osteoclastic graft resorption (C). A—Notice the sharp border between the ingrowing tissue and the graft material. At the bottom is membranous bone formation. A detail of osteoclastic graft resorption is demonstrated in part C. H&E stain; x12. B—Bone ingrowth through the two ingrowth holes. Detail of the immature woven bone is shown in part D. There was no tendency for the whole chamber to be filled with new bone. H&E stain; x12. C—H&E stain; x100. D—H&E stain; x100.
Discussion

When investigating the healing response of bone defects that have been filled with bone grafts or biomaterials, it is important to standardize the main influential factors in the restoration process. Several growth factors, signaling proteins, and bone marrow cells are known to have biological influences. Another important influential factor is the mechanical loading condition (5). In our bone chamber model, the new tissue grew into the chamber toward the investigated material perpendicular to the loading axis of the tibia. By placing the bone graft material outside the tibial loading axis, it was possible to study biological factors in the bone graft incorporation process in the absence of any interfering mechanical influences.

The only clinical complications in our study were caused by infection. There was good fixation of the chambers in the tibial bone. No skin healing problems occurred, even though skin can be expected to become more vulnerable after several repeat operations. The complication rate in our experiment (10% failure in 60 operations) was satisfactory, especially considering that each operation involved harvesting from three separate chambers. Commercially pure titanium is well tolerated as a permanent implant (6). Repeated harvesting in the same experimental animals was possible because of the properties of this material and the good perioperative conditions.

There was variation in response among animals, which resulted in fairly wide distribution of the histomorphometric measurements. Because we used three chambers per animal, interanimal variation could be eliminated by using an intra-animal control. The wide standard deviation and the small number of experimental animals jeopardized the discriminating power of our graft experiment. Increasing the number of animals or implanting a more active material might overcome this problem. Repeated sampling, using the same experimental material, will further increase the power. Another advantage of repeated harvesting is that fewer large, expensive laboratory animals need to be killed.

The two types of allograft had comparable cellular reactions, with abundant macrophages and osteoclasts. However, the response varied significantly among goats. This suggests that the allograft, although derived from only one donor goat, was not equally immunogenic in every host goat. Major histocompatibility complex antigens or the presence of necrotic tissue in the bone grafts may have been responsible (7).

The low amount of bone ingrowth into the chambers containing allograft indicates that this type of bone graft does not stimulate any clinically important bone growth activity. Stimulation of bone ingrowth could be encouraged by growth factors, such as bone morphogenetic proteins (BMP), that are released from the bone matrix during resorption. The BMP are present in minute amounts in natural bone, whereas activity for progenitor proliferation and differentiation can be expected only after administration of pharmacologic amounts of micromolar concentrations (8). The low amount of initial bone ingrowth makes this bone chamber model suitable for studying potential bone growth stimulatory agents in combination with generally applied bone graft materials.

There were no signs of osteoconduction in the specimens harvested from the chambers containing structural or chipped bone graft. However, in animal experiments in which impacted chip grafts were implanted in the femur (9) or acetabulum of the goat (10), the chips served as a scaffold for new bone apposition. It appears that for this phenomenon to occur, mechanical loading may be a prerequisite, so that micromotions are generated between the chips. On the other hand, a structural graft constitutes a stable construction in hip reconstructive surgery, with no micromotion inside the graft. Only marginal graft incorporation has been observed with this type of graft (11). The absence of mechanical loading on the structural graft implants in our experiment might explain the different histologic pattern, compared with that in human retrieval studies.

Our reusable bone chamber model enabled bone and tissue ingrowth measurements, whereas in some comparable animal models, the chambers always became entirely filled with bone tissue within a short healing period. The “bone harvest chamber” was implanted into cortical bone of rabbits to study small bone healing specimens (1). Another model, using the “analytic bone implant,” investigated the restoration on response of trabecular bone (12). In both models, bone recovery was already at its maximum within a short time span. Therefore, the designs are not suitable for studying bone healing stimulation materials and soft tissue responses.

In conclusion, our RSBC can hold sufficient amounts of bone graft material to study tissue and bone ingrowth under controlled conditions. The chamber was designed for repeated experiments in the same animal, thus lowering intersample variability and the number of the laboratory animals required.

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References


