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# Soft Tissue Movement and Stress Shielding Do Not Affect Bone Ingrowth in the Bone Conduction Chamber

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**A variety of bone chambers are used in orthopedic research to study bone and tissue ingrowth in small and large animals. If different bone chambers are placed in one species, differences in bone ingrowth are observed. For instance, bone ingrowth in the bone conduction chamber (BCC) is high, but is low or absent in the repeated sampling bone chamber (RSBC).**

**This difference may be explained by the design and fixation of these chambers. It is known that stress shielding and micromovement can influence bone formation. The objective of the study reported here was to determine whether stress shielding or soft tissue movement affected bone ingrowth in the BCC in the goat. Two types of caps were made, with fixation similar to that of the fixation plate of the RSBC. By placing the caps over the BCCs and fixating the caps directly to the tibial bone, the effect of stress shielding was studied. One cap was in direct contact with the bone chamber underneath, the other cap did not touch the chamber. This difference was used to observe whether movement of the soft tissue on top of the chamber and cap would affect bone ingrowth. Each limb received one control chamber without a cap and a chamber with a cap, either with or without contacting the BCC, yielding four implants per goat. After 12 weeks, bone and total tissue ingrowths were measured.**

**Bone ingrowth was seen in 38 of 40 chambers. Total tissue and bone ingrowths were comparable between control chambers and BCCs with a cap, irrespective of type. Neither stress shielding, nor lack of movement of soft tissue affected bone ingrowth. Other factors in the design of the chambers were responsible for the difference in bone ingrowth between the BCC and the RSBC.**

Bone chambers are often used in orthopedic research. An inner canal or ingrowth space is present in most of these bone chambers. This separate environment makes it possible to study bone and tissue ingrowth without disturbing effects from the direct environment. Additionally, the inner space of the chamber can be filled with bone, and effects of growth factors or other ways of bone treatment on bone ingrowth can be studied (1-6). A variety of bone chambers have been used in small and large animals (7-11). If the same bone chamber is used in different species, it results in a comparable response in bone ingrowth (12). Thus, the difference in bone ingrowth among species is low. On the other hand, if different bone chambers are used in one species, variable bone ingrowth is observed. For instance, the bone conduction chamber (BCC) originally developed for implantation in rats (13) was associated with considerable amounts of bone ingrowth in goats after six and 12 weeks (12). However, bone ingrowth in the repeated sampling bone chamber (RSBC) is low or even absent when placed in goats (11). Considerable bone ingrowth in the RSBC is only observed if the chamber is filled with bone graft enriched with growth factors (14).

Why bone ingrowth is high in the BCC and low in the RSBC is not known. One explanation for this discrepancy in bone ingrowth can be a difference in fixation into the bone. The BCC consists of three parts (Fig. 1A), and may therefore be less rigid than the RSBC that consists of five parts, including an inner and an outer chamber (Fig. 2A). The BCC is directly screwed into the bone, whereas one part of the outer housing of the RSBC is positioned into the bone and the other part is rigidly fixed onto the bone by use of two screws. In a pilot study in goats, BCCs placed in the trabecular bone of the proximal portion of the tibia came loose (unpublished results). Because the BCC is less rigid, it might be more susceptible to micromovement caused by the soft tissue on top of the chamber. This micromovement may affect bone growth. Another difference in design is the fixation plate that is present in the RSBC, but absent in the BCC. This plate might result in stress shielding of the inner housing of the RSBC. Since stress shielding is known to result in lower bone formation or even bone resorption, it could explain the low bone ingrowth into the RSBC (15).

This led to the following two-part question: does stress shielding and the protection of soft tissue movement of the BCC result in a lower bone ingrowth in this chamber? To answer the question, four empty bone chambers were placed in the tibia of 10 goats. Each side received one chamber with a cap and one without. Two different caps were made to place over the chambers: one in direct contact with the chamber, and the other without touching the chamber so that it would not be affected by movements of the soft tissue. To provide stress shielding, these caps were fixed on the bone by use of screws. After 12 weeks, bone

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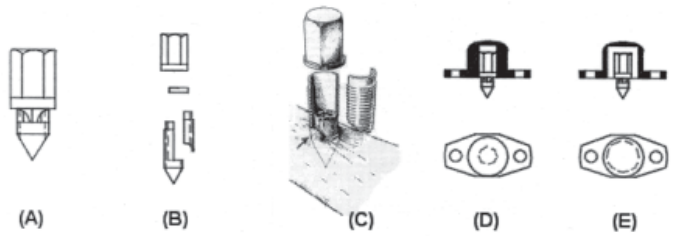


**Figure 1.** The bone conduction chamber (BCC) with a contact and a non-contact cap. The BCC (A) consists of two half cylinders (B) held together by a screwcap (modified from Aspenberg et al. 1993). A disk is used to lower the two ingrowth openings just below the tibial cortex (C). New bone and tissue ingrowth into the inner space of the chamber is possible via the marrow (arrow). Contact cap (D) and non-contact cap (E).

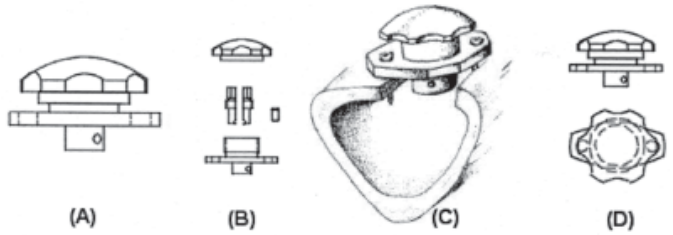


**Figure 2.** The repeated sampling bone chamber (RSBC) consists of an inner chamber (A). Two halves are placed together to make an ingrowth canal, then are screwed into the outer housing (B). An inner peg prevents rotation of the inner chamber. The entire chamber is closed with a screwcap. The outer chamber is fixed onto the bone by use of two screws in its plate. Ingrowth holes in the outer and inner chambers make tissue ingrowth possible into the canal of the inner chamber (C). The ingrowth openings are situated just below the cortex. The fixation plate of the RSBC is comparable to that of the caps used in this experiment (D) (14).

and tissue ingrowth were measured and histologic examination was carried out in routine manner.

## Materials and Methods

**Implants.** The BCC consists of two threaded half cylinders that are held together with a hexagonal closed screw cap, all made of pure titanium (Fig. 1A and 1B). The three parts are put together and screwed into the bone. Bone and fibrous tissue can grow in through two ingrowth openings at the bottom of the chamber. The chamber was designed for use in rats (13). Since the tibial cortex in goats is thicker than that in rats, we adjusted the BCC for use in goats. A 1-mm titanium disk was placed in the screwcap to provide that the ingrowth holes are located subcortically when the bone chamber is screwed into the bone (Fig. 1C). The inner dimension of the BCC is 7.5 mm long and 2 mm in diameter. The outer diameter is 3 mm. The overall length of the chamber is 13 mm. Two types of caps were made (Fig. 1D and E). To avoid mechanical stimuli reaching the inside of the bone chamber, we constructed a cap that was placed exactly over the BCC, further referred to as the contact cap. The plate of this cap was fixed onto the bone, using two screws, to provide a rigid cross-link between the bone and the plate. This plate is similar in size and shape to that of the RSBC and is fixed in similar manner onto the bone (Fig. 1D and 1E and 2C). However, movement of the skin and muscles might result in motion of this rigid cap and the chamber underneath. Therefore, a second cap was made that could be placed over the BCC without touching the chamber. This type of cap is further referred to as the non-contact cap. The caps were placed over the screwcap of the BCC and fixed onto the cortical bone, using two screws.

**Experimental design.** Ten mature, healthy, female Dutch milk goats (*Capra hircus Sana*) were obtained from the central animal department of the University of Nijmegen and were tested for caseous lymphadenitis and paratuberculosis. The goats (44 to 72 kg) received two BCCs in the cortical bone of each proximomedial portion of the tibia. The proximal chamber in each limb received either a contact or non-contact cap. The distal chamber without a cap served as a control. The side to receive a contact or a non-contact cap was chosen at random. Animals were allowed unrestricted movement in their cage and had ad libitum access to water and food. If the goats' limbs were completely loading and wound healing was normal, goats were transferred to the farm of the central animal department after two weeks. For the remaining period, they were housed in a group with other goats, with ad libitum access to water and food. All procedures were approved by the animal welfare committee.

**Surgical procedure.** Anesthesia was induced with pentobarbital (Narcovet [60 mg/ml], Apharmo, Duiven, The Netherlands) at a dosage of 0.5 ml/kg of body weight. After intubation of the goats, anesthesia was maintained, using halothane and oxygen in a semiclosed ventilation system. The hair on the distal portion of the hind limb was shaved, and the skin was washed, treated with an iodine-containing compound, and covered with sterile cloths. A longitudinal incision was made in the skin and fascia over the medial side of the proximal portion of the tibia. A hole was drilled through the medial aspect of the cortex at approximately 4 cm from the joint cleft, using a 3.1-mm drill. The hole was tapped, and the BCC was screwed in manually. A second BCC was placed similarly, 15 mm distal to the first chamber. A contact or non-contact cap was placed over the first BCC and fixed diagonally onto the cortical bone by use of two screws. The fascia and skin were closed separately with resorbable sutures (Vicryl 2-0, Ethicon, Amersfoort, The Netherlands), and the entire procedure was repeated on the opposite limb. Goats were given antibiotics for eight days (Albupen LA [100 mg/ml], Mycofarm, Cuijk, The Netherlands, 7.5 ml/d).

**Histologic and histomorphometric examinations.** After 12 weeks, animals were killed by administration of an overdose of pentobarbital. The tibias were removed, and the BCCs with surrounding cortex were fixed in buffered 4% formaldehyde. After one day, the entire contents were carefully removed from each chamber. Specimens were further fixed and dehydrated in ethanol. After plastic embedding in polymethylmetacrylate, non-decalcified, 7-µm-thick sections were taken the longitudinal axis of the specimens and were treated with hematoxylin and eosin and Goldner-Masson stains for histologic examination in routine manner. Histomorphometry was performed blindly, using a custom-made computer program (R van der Venne, Orthopedic Research Laboratory, UMC Nijmegen, The Netherlands), and obtaining digitalized pictures of the sections. A measuring rod was used for calibration, and true values were calculated by transforming the measured pixels to the known distance. Area of bone ingrowth (in square millimeters), area of total tissue ingrowth (in square millimeters), and width of specimen (in millimeters) were scored blindly in three sections per chamber, each at a distance of 140 µm from the next (Fig. 3). The bone ingrowth distance was calculated by dividing the area of bone in-

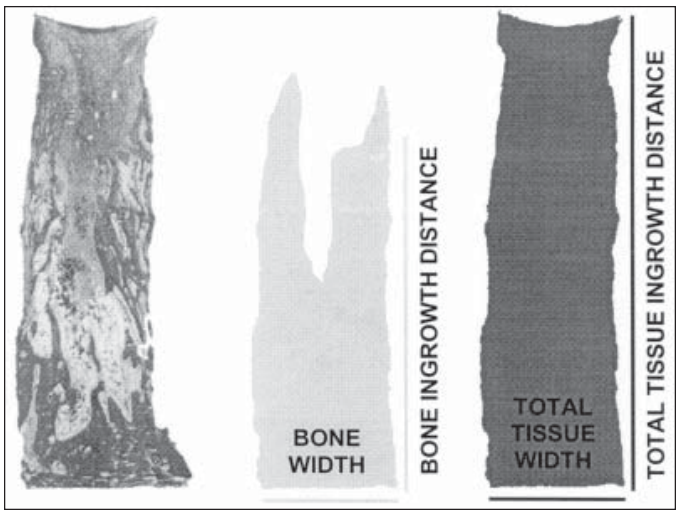


**Figure 3.** Results of histologic examination of a specimen obtained from the bone conduction chamber after 12 weeks (A). The area of bone ingrowth is divided by the width of the specimen, resulting in the bone ingrowth distance (B). The area of total tissue ingrowth is divided by the width of the specimen, resulting in the tissue ingrowth distance (C).

growth by the width of the specimen. Dividing the area of total tissue ingrowth by the width of the specimen resulted in the tissue ingrowth distance, including marrow cavities and bone.

**Statistical analysis.** A two-way analysis of variance was performed with the factors goat and chamber type (chamber with a contact or non-contact cap, or a left or right control chamber without a cap) to determine whether placement of a cap affected bone and tissue ingrowth. The control groups were taken as two separate groups into the analysis, divided by the type of cap for which it was a control. Furthermore, subanalysis was performed for the control chambers to determine whether there was a difference between left- or right-placed chambers.

## Results

**Clinical evaluation.** All goats were standing within one day after surgery, and had normal gait pattern within one week. Normal wound healing was seen in all goats, and infections did not develop. At death, all chambers were strongly fixed into the bone.

**Histologic analysis.** Two control chambers without a cap did not have bone formation. Bone ingrowth was observed in all other chambers. A layer of fibrous tissue, variable in thickness, always preceded bone ingrowth into the chamber. Bone trabeculae with marrow cavities were seen in most goats, and bone structure was normal (Fig. 3A).

**Histomorphometry.** The positioning of the control chambers, either left or right, did not affect bone or total tissue ingrowth ($P = 0.67$ and $P = 0.38$, respectively; Fig. 4). Even if the two chambers without bone ingrowth were regarded as missing values, comparable results were found. Chambers with the contact cap, as well as chambers with the non-contact cap had bone and tissue ingrowth ($P = 0.57$ and $P = 0.78$, respectively), similar to that for the control groups without a cap. There was no difference in bone or tissue ingrowth between chambers with a non-contact cap, compared with the chambers with a contact cap.

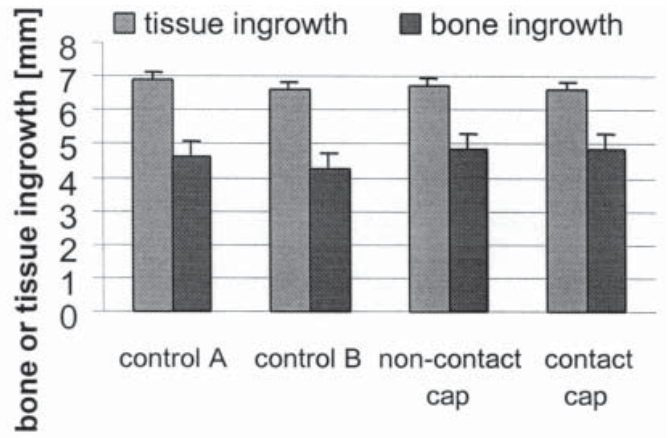


**Figure 4.** Histomorphometric results of bone and total tissue ingrowth for control chambers and chambers with non-contact and contact caps. Control A are chambers without a cap placed distally to the chambers with a non-contact cap, and control B are chambers without a cap placed distally to the chambers with a contact cap. Data are expressed as mean and SEM; n = 10 goats.

## Discussion

In general, most types of bone chambers are implanted in loaded bone. Mechanical loading conditions are important in bone healing and remodeling, in the bone chamber (16) and in the human skeleton (17). So far, it is not known whether load outside the bone chamber affects the bone formation process inside the chamber. The objective of this study was to detect a possible effect of tissue movement or stress shielding on bone ingrowth inside the BCC.

The different bone ingrowth in the BCC and the RSBC when placed in the same species (11, 12), could be caused by differences in mechanical stimuli related to differences in chamber design. The stiff and compact outer housing of the RSBC might protect the surrounding bone and the inner chamber against mechanical stimuli. In contrast, the BCC consists of two thin titanium walls (at the level of the cap), and possibly, some mechanical stimuli are involved in the bone forming process inside this chamber. On the basis of the differences in design and the differences in bone ingrowth between the bone chambers, we hypothesized that, in the BCC, the bone formation process is influenced by mechanical stimuli outside the chamber, either by stress shielding or by soft tissue movement. Caps were placed over the chambers to provide stress shielding of the BCC. The non-contact cap was made to rule out the possibility that the surrounding soft tissue would result in motion of the chamber.

Because the caps are comparable in size and fixation to that of the outer chamber of the RSBC (Fig. 1 and 2), we expected different bone ingrowth if a cap was placed over the BCC. However, differences were not observed for bone and fibrous tissue ingrowth between chambers with or without a cap, either loose or tight-fitting.

If stress shielding is not the reason for the difference in bone ingrowth, other factors are involved that govern bone and tissue ingrowth. In many bone chamber studies, the first harvest is discarded to let the implant get fully osseo-integrated, as well as to rule out the effects of trauma. With use of the BCC, it is not possible to discard the first harvest. When placing the chambers into the bone, the surrounding tissue is traumatized. This stimulates production of local trauma factors, which

spread into the space and stimulate bone ingrowth. These factors can only reach a limited distance into the chamber, and consequently, bone ingrowth will stop at a certain point. This distance can be important in the bone formation process. The RSBC consists of an inner core that fits into the outer chamber, resulting in thicker walls and, thus, greater length of the ingrowth openings (1.6 mm for the RSBC and 0.5 mm for the BCC). Also, the inner diameter (3 mm in the RSBC versus 2 mm in the BCC) and, thus, the distance from one ingrowth hole to the other differ. The ingrowing bone or fibrous tissue has to overcome a larger distance in the RSBC before reaching the inside of the chamber, compared with that of the BCC.

If only the distance would disturb the process of bone formation, it also would take place in the RSBC, but at a slower rate. Besides the distance, the shape of the ingrowth holes is important for optimal bone ingrowth. Although the cross-sectional area is 2.25 $mm^2$ in both chambers, the shape is different. The BCC has two quarter-circle shaped ingrowth openings at the bottom of the chamber (radius of 1.5 mm), whereas in the RSBC, the outer housing and the inner core have two round ingrowth holes (1.5 mm in diameter). In a study in which the micromotion chamber was used, square ingrowth holes were found to have more fibrous tissue differentiated into bone than did round ingrowth holes after 20 cycles of micromotion (0.5 mm in amplitude in 30 sec) per day for a period of three weeks. (7) Higher micromotion (0.75 mm in amplitude) resulted in lower bone formation, despite the square ingrowth holes (8). Those results indicate that small changes in the shape of ingrowth holes and in the range of micromotion can have a large influence on bone ingrowth.

It seems unlikely that use of a larger number of animals would have changed the final outcome. Three sections were measured for each chamber, with a standard error value < 0.2 mm. The inter-animal variation was larger than the intra-animal variation (i.e., the difference in placement of the chamber [left or right]). The positioning of the control chambers without a cap did not differ between left or right; thus, an effect of location does not seem to influence the results. Because of the large variation between goats, a difference of at least 1 mm is required to find an effect of cap placement. A previous study of BCCs in the goat indicated considerable bone ingrowth at 12 weeks, and the bone was more mature than that at six weeks (12). Hence, the 12-week period enhanced the discriminative effects of the parameters on bone ingrowth in the BCC.

In conclusion, we did not find lower bone ingrowth in BCCs with a cap, compared with control chambers without a cap. Stress shielding and soft tissue movement did not affect bone ingrowth in BCCs. The lower bone ingrowth that was previously observed in RSBCs, compared with BCCs, may be related to other differences in the chamber design.

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