# Cement particles inhibit bone growth into titanium chambers implanted in the rabbit

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**Particles of bone cement have been shown previously to stimulate the resorption of bone. The purpose of this study was to determine whether particles of bone cement (BC) have an adverse effect on bone ingrowth. The bone harvest chamber was implanted bilaterally in the proximal tibial metaphysis of 6 mature rabbits. Both the fixed outer cylinder and the inner removable core of the chamber have a transverse 1 mm wide pore providing a continuous canal for tissue ingrowth. After an initial 6-week period for osseointegration of the outer cylinder, the contents of the inner core were harvested repeatedly at 3 weekly intervals. In the first series of rabbits, the carrier solution, 1% sodium hyaluronate (Healon) was implanted first. In subsequent implantations, Healon was mixed with small fabricated particles of BC (averaging 3.54 mm in diameter) to fill the channel of the core. The contralateral chamber was left empty and served as a control. In the second series of rabbits, implantation was carried out sequentially using the same material bilaterally. The sections from the control harvests, and those with Healon alone contained extensive trabecular bone arranged longitudinally in the canal, in a fibrovascular stroma. The sections containing BC particles were infiltrated by foamy, mononuclear and multinuclear histiocytic cells. Less trabecular bone was seen in the sections containing BC particles compared to the control sections or those containing Healon alone. Previous studies have shown that particles of bone cement stimulate bone resorption. In this study, BC particles have also been shown to diminish the formation of new bone.**

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Long-term studies have demonstrated excellent biocompatibility of orthopedic implants stabilized with bone cement (Charnley 1970, 1979, Jasty et al. 1990). Although bulk cement appears to be well tolerated, particulate cement has been shown to evoke a florid foreign body and chronic inflammatory reaction similar to that seen around loose, cemented joint replacements in humans (Sloof 1971, Lindwer and Van Den Hoof 1975, Mirra et al. 1982, Goldring et al. 1983, Sund and Rosenquist 1983, Johanson et al. 1987, Jones and Hungerford 1987, Goodman et al. 1988, 1989, Goodman and Chin 1990). Particles of cement may be introduced during the initial implantation procedure, due to inadequate mixing of the polymer with the monomer (Goodman 1989). Cement may also undergo fatigue during intermittent loading of the arthroplasty, further generating cement debris (Jasty et al. 1991).

Particulate cement has been associated with lysis of the surrounding bone. Jones and Hungerford (1987) have coined the term "cement disease" for widespread osteolysis surrounding a cemented prosthesis. However, this process can be localized and associated with a seemingly stable prosthesis without other signs of loosening (Anthony et al. 1990, Maloney et al. 1990).

Although particulate cement has been associated with resorption of bone (Horowitz et al. 1988, Herman et al. 1989, Maloney et al. 1990, Willert et al. 1990), whether particulate cement adversely affects bone formation has not been elucidated. In this study, the authors investigate the effects of particulate cement on bone ingrowth into a single pore in the bone harvest chamber implanted in the rabbit tibia.

## Material and methods

### *The bone harvest chamber*

The bone harvest chamber is a titanium device which is implanted in the proximal tibial metaphysis of mature rabbits (Albrektsson et al. 1984; Figure 1). Both the outer cylinder and the inner removable core

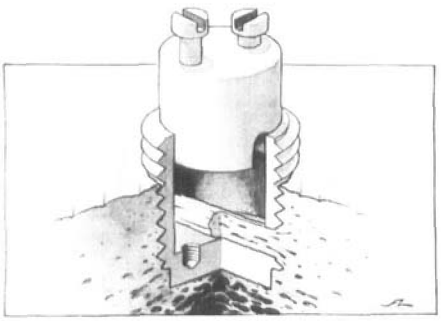


Figure 1. The bone harvest chamber.

have a transverse 1- mm wide pore for tissue ingrowth. The inner core contains a 1 × 1 × 5 mm groove that is co-axial with the holes in the outer cylinder, providing a continuous canal through the chamber for tissue ingrowth. The initial 6-week period after implantation is reserved for osseointegration of the outer cylinder within bone; thereafter, the contents of the inner core can be harvested without disturbing the cylinder or the surrounding bone.

### *Implantation procedure*

6 mature, lop-eared rabbits of both sexes were used in the first series and 6 mature, male New Zealand white rabbits were used in the second series. After induction of general anesthesia, both lower extremities were shaved from the upper thigh to the feet. Each animal was placed supine on the operating table and the lower extremities were prepared with a chlorhexidene/alcohol solution and draped to expose the proximal tibiae. Similar operations were performed bilaterally. A 3 cm incision was made over the proximal leg and the medial tibia was exposed subperiosteally. An air-powered, 6 mm diameter, hollowed drill was used to excise a round cortical window, just anterior to the insertion of the medial collateral ligament in the proximal tibia. The cylinder of the bone harvest chamber was screwed into bone with a special wrench so that the pore in the cylinder was just below the level of the outer cortex of the medial tibia. The core was then inserted into the cylinder so that the pores in the cylinder and core were co-axial. The 2 screws connecting the components were tightened. The wound was then closed in 2 layers.

2 series of experiments, with a somewhat different layout, were performed.

### *First series*

The first series was based on simultaneous harvests from bilateral chambers containing experimental and control sides. After a 6-week period of osseointegration, the contents of the chambers were harvested bilaterally for the first time. Thereafter, the Healon carrier was inserted on one side, to be compared with the "empty" chamber on the contralateral side. After 3 weeks, the chambers were harvested again; the core and the inner portion of the cylinders were cleansed with a saline soaked cotton swab to remove any remaining biomaterial. The previously untreated side was now filled with particles of cement in the Healon carrier and the other side became an "empty" control. After harvest 3 weeks later, the particles-versus-control implantation was repeated with a switch of sides, and a final harvest was done after another 3 weeks.

### *Second series*

In the second series, a bilateral implantation protocol was followed. After the first harvest at 6 weeks, a 3-week control period in which no material was implanted was followed by a harvest. Healon was then implanted bilaterally. After 3 weeks, the chambers were harvested and then cleaned as outlined above. Cement particles in the Healon carrier were then placed in the chamber bilaterally. After 3 weeks, the chambers were harvested.

### *Particulate materials*

The carrier solution for the particulate materials was 1% sodium hyaluronate (Healon, Pharmacia AB, Uppsala, Sweden). This solution was viscous enough to remain in the core acutely when the core was manipulated. For the subsequent implantations, Healon was mixed with fabricated particles of bone cement (BC) (Simplex Surgical Cement, Howmedica, Rutherford, NJ, U.S.A). The fabricated particles of bone cement were made at the Harrington Arthritis Research Center in Phoenix, AZ, U.S.A. and averaged 3.54 μm in diameter (standard deviation 6.59) using laser scatter analysis (Emmanual and Hedley 1991). In the first series, 25 mg of the particles were measured in a sterile Petri dish; 0.4 mL of Healon was added and the solution was mixed with a sterile spatula for 2 min. This yielded a concentration of $2.5 \times 10^8$ particles /mL. In the second series, a concentration of $1 \times 10^8$ particles/mL was used. The solution was aspirated with an 18-gauge needle attached to a 1-cc syringe. Air bubbles were then expelled from the syringe. The Healon/BC composite was introduced into the core via the needle and syringe to fill the groove in the core.

### *Evaluation*

By mistake, 2 of the intended carrier-versus-control pairs in the first series received Healon implanted on both sides. 1 chamber became infected after the first, and 1 after the second implantation with cement particles. These infected harvests were discarded and the animals were killed. All the animals in the second series survived without complication.

The harvested tissue was fixed in 10% buffered formalin, decalcified in Parengy's solution, dehydrated in decreasingly dilute ethanol solutions, followed by xylene and hot paraffin substitution, then embedded in paraffin blocks and cut into 5-mm sections and stained with hematoxylin and eosin. Histomorphologic analysis was performed of multiple serial, longitudinal sections, that enabled the entire length of the specimen to be visualized on one slide. The type, amount and location of bone and fibrous tissue were noted, as were the presence of a foreign body reaction (histiocytes and giant cells) and acute (polymorphonuclear leukocytes) or chronic (lymphocytes and plasma cells) inflammation. In the first series, the Videoplan system (Kontron Bildanalyse GmbH, Germany) was used to measure the percentage volume of trabecular bone in 2 entire longitudinal sections from the center of each specimen, using a magnification of 100×. The average of the 2 values was used in the analysis. It was found that the 2 values for bone ingrowth from the same specimen varied by ≤ 5 percent. Thus, in the second series, 1 longitudinal section from the center was taken for analysis of bone ingrowth, using a computerized image analysis system (Microcomp-PM, Southern Micro Instruments, Montgomeriville, PA, U.S.A.).

### *Statistics*

The statistical analysis was performed using the Kruskall-Wallis and signed rank tests using the Statview Statistical Program (Abacus Concepts, CA, U.S.A.).

## Results

### *Specimens from the initial 6-week harvest*

The specimens from the first harvest were light pink, smooth and gritty. The shape of the specimens conformed to the shape of the groove in the core. Histologically, the sections contained extensive trabecular and woven bone, covered by plump or angular osteoblasts. These cells were also embedded in round lacunae within the pink bone matrix. The bony trabecula were generally arranged parallel to the longitudinal axis of the chamber. The fibrous interstitial tissue demonstrated increased cellularity with mesenchymal cells adjacent to pink-staining, amorphous osteoid. Numerous capillaries were seen throughout the fibrous stroma. Near the periphery of the section, the trabecula were thicker. Inflammatory cells were not prominent.

### *Control, nonimplanted specimens (3 weeks)*

These specimens resembled the initial 6-week harvest.

### *Sodium hyaluronate (Healon) specimens*

All of the specimens in which Healon had been implanted contained extensive trabecular bone in a fibrovascular stroma (Figure 2). These specimens could not be distinguished from nonimplanted control specimens.

### *Sodium hyaluronate (Healon) specimens containing cement particles*

In the sections containing Healon and BC, large areas were infiltrated by foamy mononuclear and multinuclear histiocytic cells associated with round voids resembling polymethylmethacrylate particles measuring up to 30 µm in diameter; the polymethylmethacrylate is dissolved by the organic solvents during the processing of the specimens (Figure 3). Smaller particles, a few microns in diameter—probably barium sulfate which is not dissolved during processing—could also be identified; some of these particles were located within histiocytes and giant cells. In other areas, dense aggregates of histiocytic and lymphocytic cells were associated with the cement ghosts and smaller particles; this granulomatous reaction was often surrounded by a thin fibrous tissue layer. There was no evidence of acute inflammation (polymorphonuclear leukocyte infiltrates). The trabecular bone appeared to be similar in quality but less in quantity compared to the contralateral control sections or those containing Healon alone.

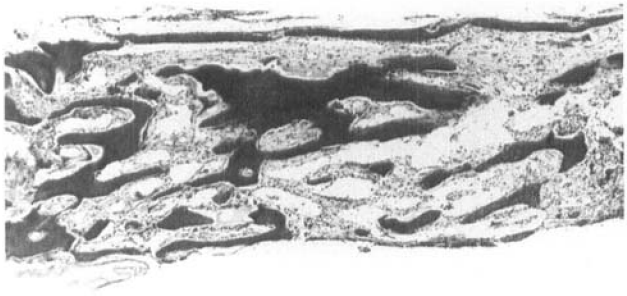
There were no differences regarding qualitative histology between the 2 series.

### *Histomorphometric analysis*

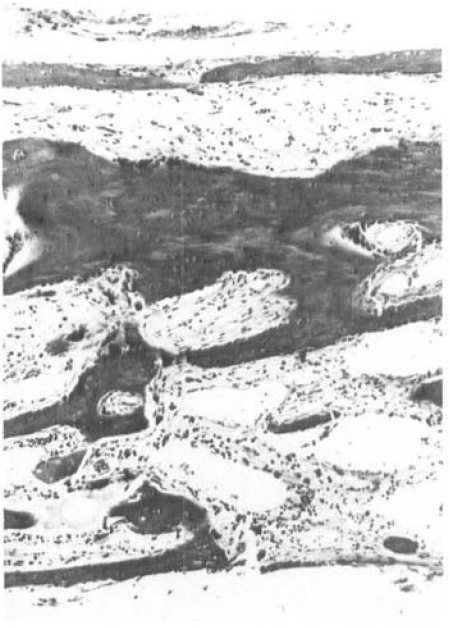
*First series.* The results in the first series are listed in Table 1 and summarized in Table 2. The percentage volume of trabecular bone for the 13 control harvests averaged 31 (SEM 2) percent, and for the 8 harvests containing Healon carrier alone was 30 (SEM 3) percent. The 9 sections with Healon plus cement particles contained 21 (SEM 2) percent bone ingrowth. If the volume taken up by the cement particles is taken into account, then the average value for bone ingrowth in the presence of Healon plus cement particles is 22 (SEM 2) percent.

If independence of harvests is assumed and the Kruskall-Wallis test is applied to the 3 groups (control,

Figure 2. Sodium hyaluronate (Healon) specimen. These photomicrographs demonstrate extensive ingrowth of trabecular and woven bone in a fibrovascular stroma. HE stain.

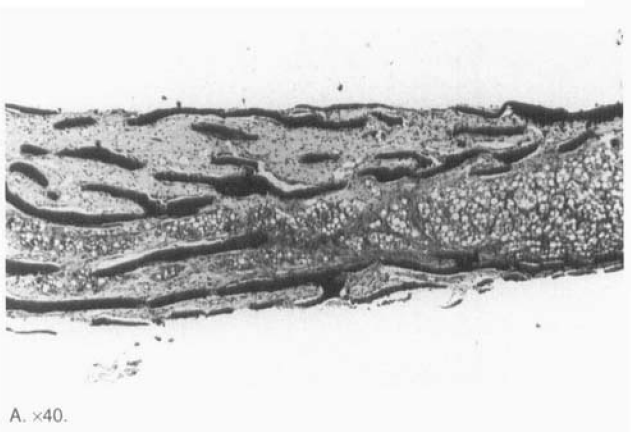


A. ×40.



B. ×100.

Figure 3. Specimen containing sodium hyaluronate (Healon) and cement particles. Large areas in this section are infiltrated by foamy mononuclear and multinuclear histiocytic cells associated with round voids resembling polymethylmethacrylate particles. Bone ingrowth is less extensive. HE stain.



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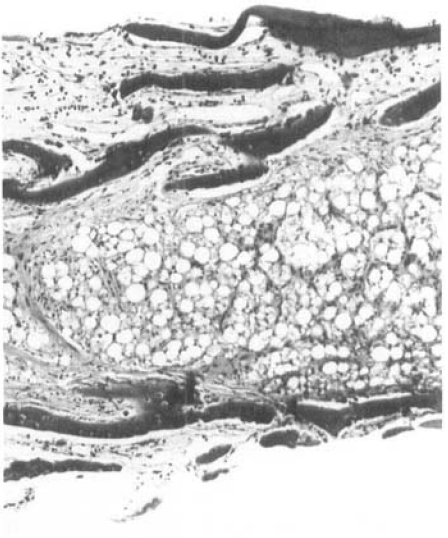

B. ×100.

Healon, Healon plus cement particles), sections containing Healon plus cement particles demonstrated less bone ingrowth compared to the other 2 groups ($P < 0.05$).

If independence of harvests is not assumed, available for analysis are 9 pairs of harvests in 5 animals with Healon plus cement particles on 1 side, and a contralateral non-implanted control. In each of these pairs, the controls yielded more bone ($P < 0.01$ using the signed rank test). It was not possible to statistically evaluate the effect of Healon carrier alone using contemporary controls, as the number of such paired harvests was only 4.

*Second series.* The results in the second series are listed in Table 3. The volume of trabecular bone for the second series was as follows: nonimplanted con-

Table 1. First series. Histomorphometric bone content (the volume of trabecular bone as a percentage of the entire volume for tissue ingrowth) of bilateral bone harvest chambers constituting experimental/control pairs. A Healon with bone cement experiment was repeated once in each animal with a switch of sides

| Rabbit no. | Healon | Controls opposite Healon | Healon with bone cement (corrected)[a] | Controls opposite bone cement |
|---|---|---|---|---|
| 1 | 42 | 26[b] | 16 | 43 |
| | | | 20 | 26 |
| 2 | 27 | 18[b] | 11 | 24 |
| | | | 24 | 30 |
| 3 | 39 | 33 | NA | NA |
| | | | NA | NA |
| 4 | 32 | 21 | 24 | 29 |
| | | | 37 | 36 |
| 5 | 29 | 42 | 20 | 29 |
| | | | 21 | 26 |
| 6 | 25 | 28 | 24 | 31 |
| | | | NA | NA |

NA not available.
[a] Healon with bone cement (corrected) accounts for the volume taken up by the cement particles.
[b] Control side treated with Healon by mistake.

Table 2. First series. Summary of the percentage volume of bone, using the bone harvest chamber

| Implant | Weeks of implantation | Number of specimens | Percent bone ingrowth *SEM* |
|---|---|---|---|
| Control side | 3 | 13 | 31 *2* |
| Healon | 3 | 8 | 30 *3* |
| Healon with bone cement (corrected)[a] | 3 | 9 | 22 *2* |

[a] See Table 1.

Table 3. Second series. Histomorphometric analysis of the percentage of bone ingrowth, using the bone harvest chamber with bilateral implantation

| Animal | Control | Healon | Healon with bone cement (corrected)[a] |
|---|---|---|---|
| 1 | 36 | 29 | 28 |
| | 30 | 36 | 24 |
| 2 | 38 | 26 | 18 |
| | 34 | 26 | 24 |
| 3 | 24 | 30 | 31 |
| | 34 | 39 | 25 |
| 4 | 37 | 24 | 21 |
| | 29 | 38 | 29 |
| 5 | 32 | 38 | 31 |
| | 32 | 40 | 23 |
| 6 | 32 | 23 | 17 |
| | 29 | 33 | 19 |
| Mean *SEM* | 32 *1* | 32 *2* | 24 *1* |

[a] See Table 1.

trols 32 (SEM 1) percent, Healon carrier alone 32 (SEM 2) percent, Healon plus cement 23 (SEM 1) percent, Healon plus cement (corrected for the volume taken up by the cement particles) 24 (SEM 1) percent. The Kruskall-Wallis test using control, Healon, and Healon plus cement groups was significant at $P < 0.01$. Intergroup comparisons showed that Healon plus cement was associated with less ingrowth of bone compared to the nonimplanted controls or Healon alone ($P < 0.01$). There was no difference in percentage of bone ingrowth between the Healon and control harvests.

## Discussion

Immediately after implantation of a cemented component, the adjacent endosteum is injured due to the surgical trauma, vascular disturbance, heat injury due to cement polymerization and toxic effects of the monomer (Sloof 1971, Haas et al. 1975, Lindwer and Van Den Hoof 1975, Rhinelander et al. 1979, Sund and Rosenquist 1983, Mjöberg 1986). The surrounding bone undergoes a regenerative process and, in so doing, prosthetic stability is established (Mjöberg 1991). Indeed, the interface between bone and the cemented implant undergoes constant remodeling in response to mechanical and biological stimuli throughout the lifetime of the prosthesis (Maloney et al. 1989).

Particles of bone cement may be implanted initially at surgery, due to inadequate mixing of the monomer

and polymer (Goodman 1989). Cement debris may also form during cyclic loading of the implant, due to fatigue fracture or abrasive wear of the cement (Jasty et al. 1991). This debris has been implicated in the resorption of bone surrounding cemented implants (Jones and Hungerford 1987, Anthony et al. 1990, Maloney et al. 1990).

This in vivo study has shown that particles of bone cement, at the concentration used in this experiment, are associated with decreased net formation of trabecular bone. This is not simply a "space-occupying effect" of the cement itself; our calculations suggest that the cement particles would occupy approximately 5 percent of the volume of the canal. Even after correction for this, the cement particles were still associated with decreased bone ingrowth. Histologically, the particles of bone cement evoke a florid foreign body and chronic inflammatory reaction. It would appear that bone ingrowth is mitigated by the space occupied by the cellular response associated with the cement particles, by the liberation of factors that reduce the formation and/or enhance the degradation of bone, or due to the toxic effect of residual monomer. The interpretation of the second series is dependent on the assumption that repeated harvests from the same chamber give reproducible amounts of bone. This has been shown to be the case: Kälebo and Jacobsson (1988) made 7 consecutive 3-week harvests in 6 rabbits with no other treatment. They found more bone at 6 weeks after implantation, probably due to the insertion trauma, but thereafter the amount of harvested bone was stable. The first harvest to be analyzed in our series was taken at 9 weeks.

During total joint replacement using cement, debris should be avoided by careful cement-mixing and surgical technique. This point may be even more important when a hybrid arthroplasty combining cemented and cementless components is considered.

## Acknowledgement

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