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Chapter 6

Effects of mechanical compression of a fibrous tissue interface on bone with or without high-density polyethylene particles in a rabbit model of prosthetic loosening

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Abstract

The mechanisms leading to aseptic loosening of a total hip replacement are not fully understood. A fibrous tissue interface can be present around the implant. Hypothetically, component micromovements can compress this interface and cause increased fluid pressure according to biphasic models. We tested the hypothesis that compression of a fibrous membrane with or without the presence of high-density polyethylene particles leads to bone degradation. A titanium implant was inserted in forty-five rabbit tibiae, and, after osseointegration was achieved, a fibrous tissue interface was generated. The animals were randomized to undergo a sham operation, treatment with compression of the fibrous membrane, treatment with high-density polyethylene particles, or treatment with both compression and particles. Morphometric analysis of the surrounding bone was performed on cryostat sections after Giemsa staining and staining of tartrate-resistant acid phosphatase activity. Forty specimens were available for analysis; five tibiae with an infection were excluded. After nine weeks, the controls showed vital bone, whereas the specimens treated with compression showed necrosis of bone and replacement of bone by cartilage in a discontinuous layer ($p < 0.05$ for both) but not fibrous tissue. Treatment with high-density polyethylene particles caused replacement of bone by fibrous tissue ($p < 0.05$) but not necrosis or cartilage formation. Compression combined with the presence of high-density polyethylene particles caused bone necrosis and loss of bone with replacement by cartilage and fibrous tissue ($p < 0.05$). In this in vivo study in rabbits, fibrous membrane compression led to bone necrosis and cartilage formation, possibly because of fluid pressure or fluid flow, whereas the presence of high-density polyethylene particles led to the loss of bone with replacement of bone by fibrous tissue. Cartilage formation may be a protective response to fluid pressure and/or fluid flow. Fibrous membrane compression may play an important role in the early stages of loosening of a total hip replacement. The findings of this study suggest that implantation techniques that prevent the formation of a fibrous tissue interface (which may act as source of fluid pressure and/or fluid flow) may be beneficial in reducing implant loosening.

Introduction

The most frequent, and often devastating, long-term complication after total hip replacement is aseptic loosening. Despite vigorous research, the precise mechanisms leading to aseptic loosening are not fully understood.

First, insufficient initial fixation or early loss of fixation have been suggested to lead to early loosening¹. This may be caused by an implant of improper size or design, an improper cementing technique, or inferior bone quality^{2,3} inducing micromovements and subsequent detachment of the component at the interface.

Second, wear particles (in particular polyethylene) originating from components⁴ and/or cement have been considered to cause bone resorption³. This particle-induced bone resorption has been reproduced in in vitro studies⁵⁻⁷ but the results of in vivo studies have not been consistent. In some in vivo studies, particle-induced osteoclastic bone resorption has been observed^{8,9}, whereas in other studies only a decrease in bone formation was seen^{10,11}.

Third, high fluid pressure has been proposed to be, at least in part, responsible for the process of loosening¹². Pressures as high as 0.1 MPa (780 mm Hg) have been measured in the pseudogjoint cavity of total hip replacements during physiological activities¹³. High intracapsular pressures are often found in loose total hip replacements^{13,14}, causing capsular distension as identified by ultrasound¹⁴. Capsular distension was less in hips that did not show clinical loosening, indicating that pressure was lower in those cases¹⁴. Peak pressures of 0.07 MPa (500 mm Hg) have been measured in the presence of pelvic osteolysis at revision surgery¹⁵. Pressure differences may induce a flow of joint fluid in the effective joint space, affecting periprosthetic bone^{3,16}.

In experiments on rabbits, an exogenously derived (oscillating) fluid pressure was shown to cause bone resorption^{17,18}. Furthermore, Van der Vis et al.¹⁹ were the first, as far as we know, to apply endogenous fluid pressure to a fibrous membrane in rabbits and they found bone resorption. Similar results have been obtained in rats²⁰. In a study in which polymethylmethacrylate particles and endogenously derived fluid pressure were administered to rats, the fluid pressure appeared to cause distinct bone resorption, whereas polymethylmethacrylate led to minimal resorption only²¹.

Radiostereometric analysis showed that the probability of late clinical loosening is increased when prosthetic components migrate soon (within two years) after implantation^{22,23}. Migration of a prosthetic component should be possible only when the component is surrounded by a membrane of fibrous tissue. Consequently, there are correlations between the presence of a fibrous membrane, migration of a prosthetic component, and late clinical loosening^{1,12}. It has been speculated that the aforementioned fibrous membrane acts as an interstitial fluid compartment^{12,19}. It has been postulated that micromovements of the prosthesis cause repetitive compression of this membrane and thereby increase local fluid pressure that may affect the integrity of the surrounding bone. Resorption and subsequent loosening may be the result. Indeed, compression of a fibrous tissue membrane induced osteoclastic bone resorption in recent experiments on rabbits and rats^{19,20}.

In the present experimental study with rabbits, an endogenously derived fibrous tissue membrane interposed between the vital tibia and a titanium surface was compressed. Such a membrane can be thought of as a biphasic model consisting of a solid matrix and an interstitial fluid compartment with physiologic properties. In this study, it was hypothesized that compression would lead to a combination of stress in the solid matrix and elevation of fluid pressure in the fluid compartment^{24,25}. Furthermore, the effect of the presence of high-density polyethylene particles was also studied, especially as to whether their presence might lead to modulation of the response to interface compression.

Materials and Methods

Animals

Forty-five skeletally mature New Zealand White Rabbits (BMI, Helmond, The Netherlands) with a mean weight (and standard deviation) of 38.20 +/- 0.19 N, were used. The protocol for the animal experiments was approved by the animal ethical committee of the Faculty of Medicine, University of Amsterdam and all animal handling was performed according to Dutch laws for treatment of research animals.

The implant

The model used in this study is a modification of the model introduced by Van der Vis et al.⁽¹⁹⁾. It consists of a cubical titanium implant that is inserted into the rabbit tibia. A fibrous tissue interface between the implant and vital bone is created, and

the effect of compression of this fibrous membrane on bone is evaluated. Our modification allows the possibility of administering particles at the interface. The implant is a cube (7.2 mm³) with a cylindrical canal (diameter, 4.50 mm) and a groove (7.2 x 2 x 2 mm) on one end. It is made of commercially pure titanium with a surface roughness of 1.8 µm. A nonmoveable “static” cylinder with a diameter of 4.50 mm and a groove of 4.45 mm x 2 mm x 2 mm fits in the canal of the implant (Fig. 1, A).

When the device is implanted in bone (see section entitled Surgical Procedures), the roof of the groove faces the cortical bone surface and both sides of the groove are embedded into the bone. In this way, a bone bridge that is 7.2 mm long, 2 mm wide and 2 mm high is created, and it is surrounded by the titanium implant on all sides except for the endosteal surface toward the bone marrow cavity. In this study, the construct was allowed to integrate into bone for five weeks. Then, the “static” cylinder was exchanged for a “dynamic” cylinder (Fig. 1, B) which had a groove on one end of 4.45 x 2 x 2.2 mm; thus, its width was 200 µm in excess of the width of the groove of the “static” cylinder. The “dynamic” cylinder also had a biconcave handle on top that could be rotated to each side with a maximum amplitude of 100 µm. A stop screw prevented further rotation. Thus, a space of 100 µm, in which particles could be administered, was created between vital bone and the titanium surface at either side of the bone bridge (see section entitled Surgical Procedures).

During the following two weeks, a 100-µm-thick fibrous layer was allowed to form in this space at either side of the standardized bone bridge in the presence or absence of particles. After the two weeks of fibrous tissue growth, the biconcave handle of the “dynamic” cylinder was grasped through the intact skin and manually rotated alternately clockwise and counterclockwise, thus rotating the cylinder inside the implant, intermittently compressing the fibrous membrane on both sides of the bone bridge (see section entitled Test Phase). By means of this movement with controlled amplitude, only the fibrous membrane was compressed without direct mechanical contact with the underlying bone.

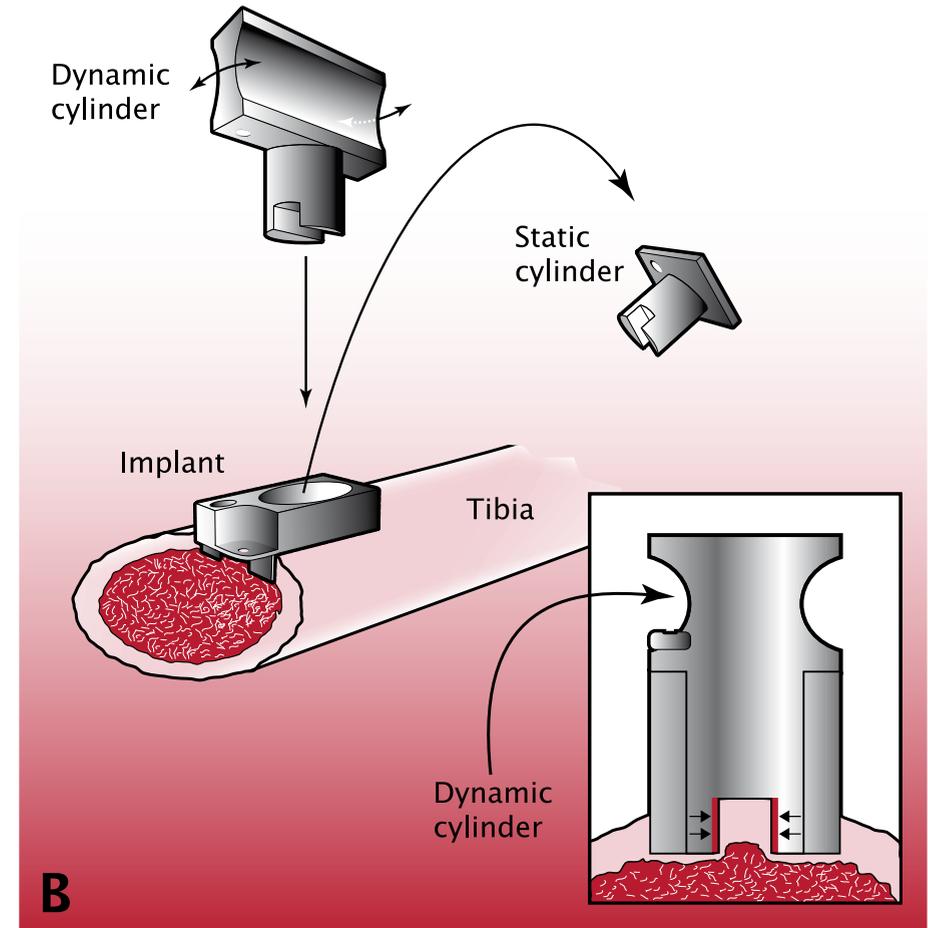
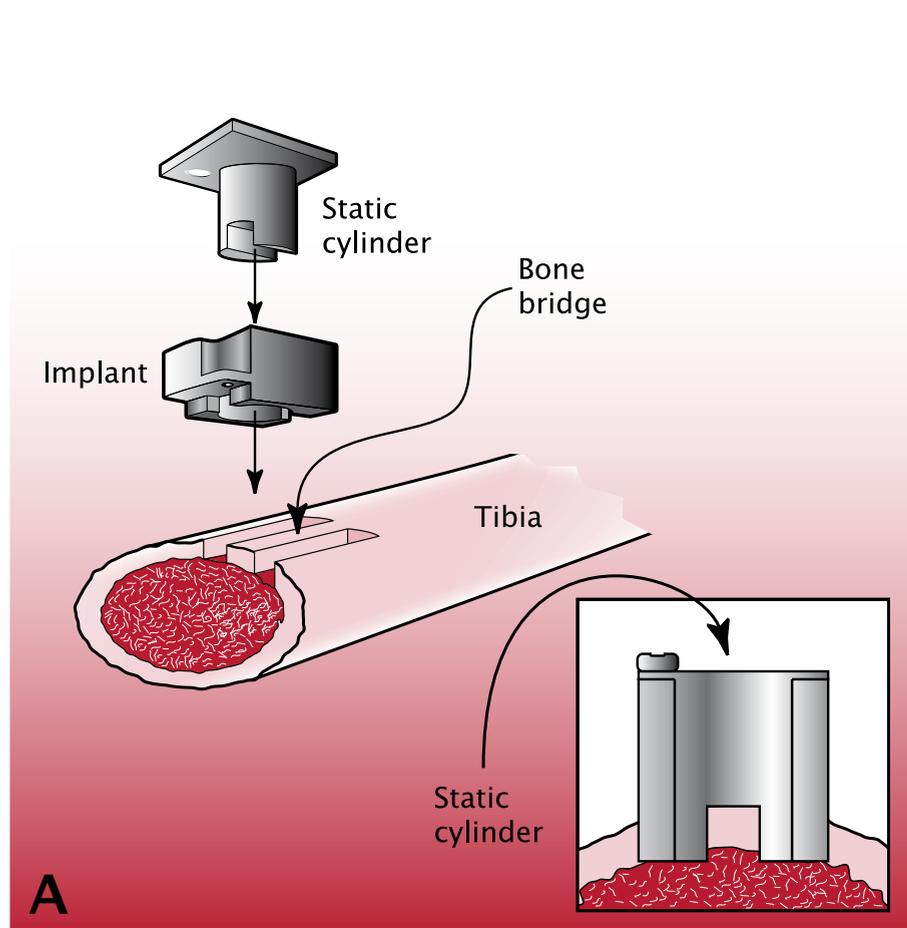


Fig. 1. Schematic drawings of a bone bridge and the implant with the “static” cylinder (**A**) and the “dynamic” cylinder (**B**). Implant dimensions are 7.2 mm³ with a cylindrical canal with a diameter of 4.50 mm. The groove on one end of the implant is 7.2 mm x 2 mm x 2 mm. The groove on one end of the static cylinder is 4.45 mm x 2 mm x 2 mm, and the groove of the dynamic cylinder 4.45 mm x 2 mm x 2.2 mm. Cross sections (inset) show the implant straddling the bone bridge in A, and with interposed fibrous tissue on both sides of the bone bridge (arrows) and bone formation towards the medullary cavity in B.

Particulate materials

The high-density polyethylene particles that we used were donated by Smith and Nephew Richards (Memphis, Tennessee, USA) and were produced by Shamrock Technologies (Newark, New Jersey, USA). They were small enough (mean diameter, 4.6 μm ; range 0.4– 8.0 μm) to be phagocytosed by macrophages²⁶. The particles were polymerized as high-density polyethylene and then were ground with a proprietary milling process into a smaller particle size. They were reported to be 100% high-density polyethylene particles and highly crystalline with a specific gravity of 0.95. The size of the particles was measured with use of a scanning electron microscope interfaced with a morphometric image analysis system (Beckham Coulter, Fullerton, California, USA). The particles were washed two times in alcohol followed by two additional washes in sterile water, and, after centrifugation, the supernatant was removed and the particles were air-dried in a sterile environment. The sterility of the particles was verified in anaerobic and aerobic cultures.

Surgical procedures

All animals had two operations under aseptic conditions. Anaesthesia was induced with 10 mg/kg xylazine (Bayer, Leverkusen, Germany) and 50 mg/kg ketamine (Aesculaap, Boxtel, The Netherlands) intramuscularly and was maintained with inhalation anaesthesia with a mixture of isoflurane, nitrous oxide, and oxygen. Antibiotic prophylaxis with use of 10 mg/kg of enrofloxacin (Bayer) administered subcutaneously was started one day before the operation and was continued until one day after the operation.

In each animal, the operations were performed on the right tibia. In the first operation, a medial parapatellar approach was used to expose the metaphyseal bone surface of the tibia. Onto this relatively flat area, a metal template with two longitudinal slots was fixed with two cortical screws (diameter, 1.45 mm; Mathys, Bettlach, Switzerland). The dimensions of the slots corresponded with the bars next to the groove of the implant. Cortical bone was removed with a water-cooled burr through the open slots. In that way, a bone bridge was created with the dimensions of the groove of the implant. Next, the template was removed and the implant was inserted over the bone bridge and was fixed with two reinserted cortical screws with use of the same holes as the ones used for the template. Then the “static” cylinder was inserted through the cylindrical canal and fixed to the implant with a screw. Finally, the skin was closed over the entire device with interrupted mattress sutures (Vicryl 3-0; Ethicon, Norderstedt, Germany). The animals received 0.05 mg/kg

buprenorphine subcutaneously twice a day for two days as postoperative analgesia. As noted previously, the implants were allowed to integrate into bone for five weeks. During this period, the animals did not seem to be hampered by the implant. The animals were randomized into five groups of nine animals each. Group I consisted of the interface controls (to identify fibrous membrane formation); group II, the controls that received no treatment; group III, those treated with compression only; group IV, those that received particles only; and group V, those treated with compression and particles.

After five weeks, a second operation was performed on all animals. First, the screw was removed and then the “static” cylinder, with great care, was lifted in a vertical direction only, avoiding rotational movement that could damage the bone bridge. Then, the “dynamic” cylinder with the wider groove was inserted into the cylindrical canal of the implant thus creating the space of 100 μm on either side of the standardized vital bone bridge and allowing the formation of a 100- μm -thick fibrous membrane during the following two weeks. In animals in group IV (particles only) and group V (compression and particles), approximately 2.5 mg of high-density polyethylene particles (the equivalent of 0.5×10^8 particles) were administered at either side of the bone bridge. This load of particles was chosen to ensure that the bone tissue was exposed to an adequate number of particles, in light of the findings in a previous report that described the loads of particulate wear debris found in periprosthetic tissue retrieved from loosened total hip replacements²⁷. A stop screw secured the cylinder to the implant, restricting the cylinder from rotating.

Test phase

The application of membrane compression by movement in group III (compression only) and group V (compression and particles) was started at seven weeks after the first operation. The biconcave handle that protruded underneath the intact skin was grasped between thumb and index finger, and the rabbit leg was held in place with the other hand. The handle was rotated until firm resistance was encountered and the stop screw prevented further rotation; then the direction of rotation was reversed. Movement was applied twice a day, sixty times during two minutes, for fourteen days (Table I). During the application of movement, sedation of the animals was not necessary.

Time	Intervention	All Animals	I Interface Control N= 9	II Control N= 9	III Compression N= 9	IV Particles N= 9	V Compression and particles N= 9
Day 0	First operation: implant with "static" cylinder placed in right tibia	45	–	–	–	–	–
Day 1 up to week 5	Osseointegration of implant and randomization into groups		9	9	9	9	9
End of week 5	Second operation: exchange of "static" for "dynamic" cylinder		9	9	9	–	–
	Second operation: exchange of "static" for "dynamic" cylinder with administration of high-density polyethylene particles		–	–	–	9	9
Week 6 to week 7	Allowing fibrous membrane formation		9	9	9	–	–
	Allowing fibrous membrane formation and high-density polyethylene particles in situ		–	–	–	9	9
End of week 7	Killing of animals		9	–	–	–	–
Week 8 up to week 9	Application of movement		–	–	9	–	–
	High-density polyethylene particles in situ		–	–	–	9	–
	Application of movement with high-density polyethylene particles in situ		–	–	–	–	9
End of week 9	Killing of animals		–	9	9	9	9

Table 1. Time course for the five treatment groups.

Processing of specimens

The animals in group I (the interface controls) were killed with an overdose of pentobarbital (60 mg/kg) at seven weeks after the first operation, and the animals in the other groups were killed in the same manner at nine weeks. The implants in the animals in group III (compression only) and group V (compression and particles), which had undergone movement, were checked to determine whether the cylinder had rotated properly. After removal of all soft tissues that covered the implant, the implant was cleaned of any bone overgrowth and the "dynamic" cylinder was removed. Next, the implant was carefully separated from the bone with the use of a chisel. Finally, the entire proximal metaphysis was removed with an oscillating saw.

The specimens were immediately imbedded in 8% gelatin white (Sigma, St. Louis MO, USA) and were slowly frozen in liquid nitrogen²⁸. Undecalcified cryostat sections (8 µm thick) were cut parallel to the surface of the bone bridges with the use of adhesive tape (Scotch tape 800; 3M, St. Paul, Minnesota, USA) to maintain the integrity of the sections²⁸. The sections were cut with the use of a tungsten-carbide-tipped knife (Spikker, Zevenaar, The Netherlands) and were stained with Giemsa (Merck, Darmstadt, Germany) for morphological orientation. Then, the pieces of tape to which the sections were adherent were cut out and mounted between two layers of glycerol jelly²⁸. For each bone bridge, three sections at different levels were selected for analysis: one was as close to the periosteal surface of the bone bridge as possible; another, as close to the endosteal surface as possible; and one, at approximately the middle of the cortex. Adjacent to the latter section, an additional section was used for localization of tartrate-resistant acid phosphatase (TRAP) activity to stain osteoclasts selectively and to establish the presence or absence of necrotic bone. Necrotic bone was characterized by the absence of cells containing TRAP activity in (bone) lacunae.

Spatially calibrated digital images were obtained by scanning an entire section using a 35-mm slide scanner (Coolscan 1000; Nikon, Tokyo, Japan). Interactively, tissue components, i.e., fibrous tissue, cartilage, and areas with absent TRAP activity, were delineated and segmented with use of the image processing program Object-Image²⁹.

Morphometric Analysis

All nine bone bridges from each of the five treatment groups were examined. TRAP activity was classified as present when cells stained brightly red and as absent when staining was hardly detectable or when cells did not stain at all. The regions of the bone bridges that contained cells without activity were qualitatively determined and labeled as necrotic. Thereafter, these areas were determined quantitatively with use of image analysis. Areas of cartilage and fibrous tissue were determined quantitatively with use of image analysis of the Giemsa-stained sections. Areas of necrosis, cartilage, and fibrous tissue were expressed as a percentage of the surface area of each bone bridge. The surface area was defined as the area of the bone bridge that had been subjected to the effect of membrane compression and/or particles, i.e., the area enclosed by both sides of the groove of the “dynamic” cylinder. Similar to the shape of the entire bone bridge, the shape of the surface area was rectangular. The median surface area for the bone bridges that were tested was 8.91 mm² (range, 8.85 to 9.04 mm²), corresponding with the length (4.45 mm) and the width (2 mm) of the groove of the “static” cylinder.

The median values of the percentages of the tissue areas were calculated. The median values between groups were compared and analyzed. The nonparametric Mann-Whitney test (release 11.0; SPSS, Chicago, Illinois, USA) was used for statistical analysis; the level of significance was set at $p < 0.05$.

Results

Morphology

After five weeks, when the “static” cylinder was exchanged during the second operation, macroscopic inspection of the inner surface of the groove of the “static” cylinder never showed adhesion of (fibrous) tissue.

At the time that the animals were killed and the implants were removed, forty of the forty-five specimens showed macroscopic apposition of bone around the implant. After removal of this tissue, it appeared that the area between the outer surface of the implant and metaphyseal bone was tightly sealed and there were no signs of inflammation. Bone marrow cavities were sealed off from the bone bridge and interface area by the formation of new bone on the groove side of the implant, thereby providing a closed system in all cases (as in Fig. 1, B, inset). Sections of these bone bridges showed vital bone and bridge edges were sharp and regular.

These observations indicated that osseointegration had occurred and inflammation was absent. Therefore, these forty specimens were included in the study (Table II). The other five animals had an abscess develop over the implant because of a bacterial infection, as proved on culture, and these specimens were excluded.

Outcome	I Interface Control N = 8	II Control N = 6	III Com- pression N = 9	IV Particles N = 8	V Com- pression and particles N=9
Cartilage					
Median percentage of surface area of bone bridge (range)	0 (0-0.16)	0 (0-0.10)	2.82* (1.06-4.68)	0 (0-0.09)	3.03*† (0.81-6.97)
Median surface area (mm ²)	0	0	0.25	0	0.27
Necrosis					
Median percentage of surface area of bone bridge (range)	0 (0-4.16)	0 (0-4.73)	14.25* (6.0-24.28)	0.82 (0-9.06)	14.60*‡ (6.14-21.81)
Median surface area (mm ²)	0	0	3.38	0.19	3.46
Fibrous tissue					
Median percentage of surface area of bone bridge (range)	0.29 (0-4.20)	0 (0-1.02)	0.77 (0-4.79)	6.16* (0.51-27.39)	3.62*§ (1.52-9.48)
Median surface area (mm ²)	0.03	0	0.07	0.55	0.33

Table II. Morphometric findings for the five treatment groups. *The difference between the treatment and groups I and II with respect to cartilage, necrosis, or fibrous tissue was significant (Mann-Whitney test, $p < 0.05$).

† The difference between group III and group V with respect to cartilage was not significant (Mann-Whitney test, $p = 0.40$).

‡ The difference between group III and group V with respect to necrosis was not significant (Mann-Whitney test, $p = 0.76$).

§ The difference between groups IV and group V with respect to fibrous tissue was not significant ($p = 0.34$).

All specimens in group I (the interface controls) showed a vital bone bridge as described above, with a thin fibrous membrane on both sides containing cells and extracellular matrix. Occasionally, cartilage was present. The thickness of the fibrous membranes was 100 µm, indicating that the space created by the broader groove at each side of the bone bridge had been completely filled with a membrane of fibrous tissue (Fig. 2, A).

Group II (controls) showed vital bone in the bone bridges as well, but, instead of an outer fibrous membrane, thin lamellae of new bone with osteocytes and areas of osteoid, with a thickness of 100 µm, were observed to have formed, (Fig. 2, B).

Only small amounts of fibrous tissue within the bone bridge were observed in group III, in which the fibrous membrane had been compressed. Conversely, larger areas of bone loss, with replacement by cartilage, were seen (Fig. 2, C). These changes were most obvious at all four corners of the rectangular surface areas where the amplitude of the movement of the “dynamic” cylinder was maximal and compression of the fibrous layer had been highest because of the design of the implant (Fig. 2, C).

Underneath the fibrous membrane, again especially at all four corners of the rectangular surface areas where membrane compression was highest, there were regions of bone tissue, including bone lacunae, that lacked cells with TRAP activity, indicating that these regions of bone tissue were not vital. In the areas of bone bridges where compression was lowest (in the middle of the rectangular surface areas), the numbers of cells showing TRAP activity in bone and lacunae were normal, indicating that bone was vital (Fig. 2, D). This pattern was consistent in all sections investigated. Areas of vital and nonvital bone were also sharply demarcated in all sections.

After membrane compression, sections of bone taken at the level of the endosteal surface demonstrated the formation of new lamellar bone. Morphometric analysis showed that bone bridges were qualitatively thicker in compressed specimens than in non-compressed specimens (approximately 1 mm compared with 0.5 mm) because of this new bone formation. However, the new bone formation occurred outside the area of analysis and therefore was not included in the study.

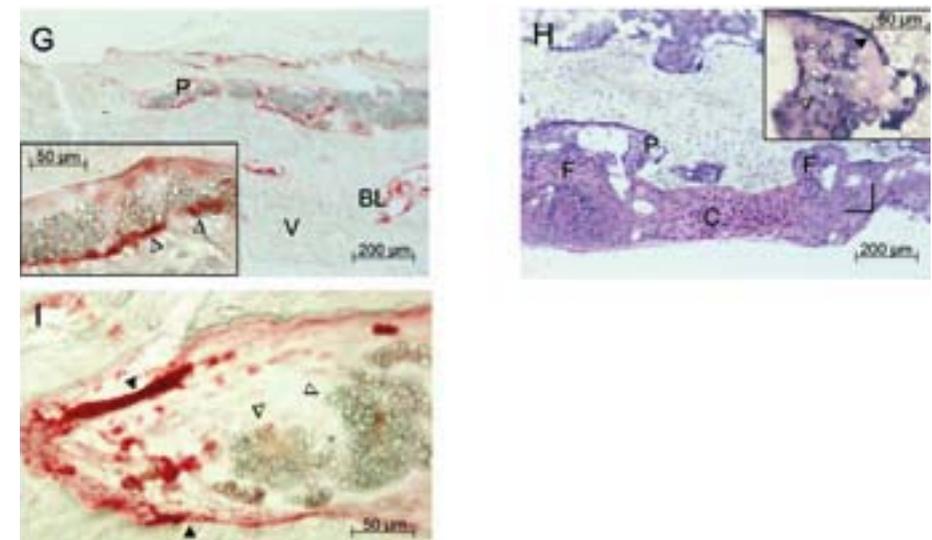
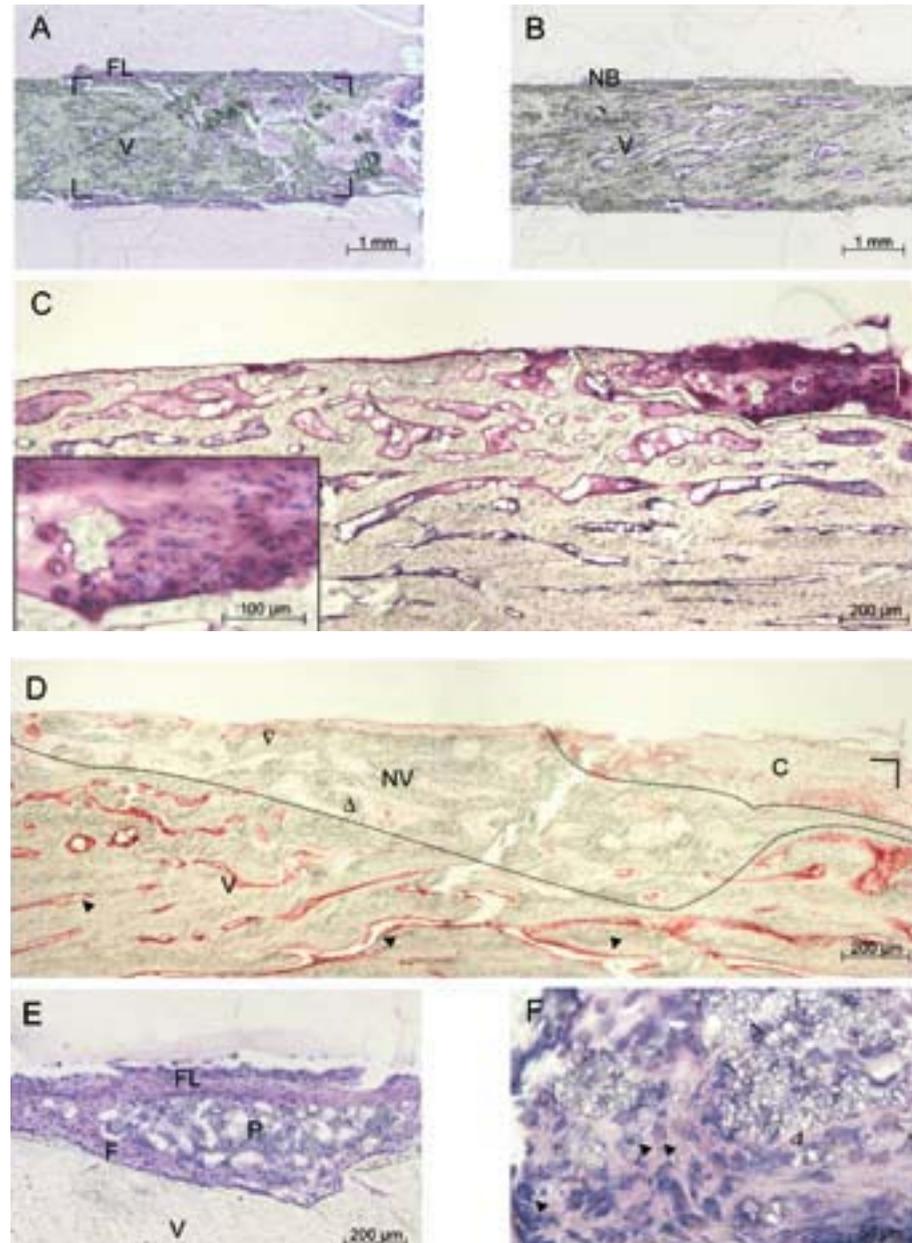


Fig. 2 Photomicrographs of sections from bone bridges in group I, the interface controls (A); group II, the controls (B); Group III, which was treated with mechanical compression (C and D); group IV, which was treated with high-density polyethylene particles (E, F and G); and Group V, which was treated with a combination of mechanical compression and high-density polyethylene particles (H and I).

A: Photomicrograph of a specimen from a bone bridge in group I showing vital bone (V) with a fibrous layer (FL) on both sides of 100 μ m. The rectangular surface area is the area enclosed by both sides of the groove of the “dynamic” cylinder; its four corners are indicated by (┌, ┐, └, ┘) (Giemsa staining, x11). B: Photomicrograph of a section from group II showing vital bone (V) with thin lamellae of new bone (NB) on both sides with osteocytes and areas of osteoid, which have replaced the fibrous layer (Giemsa staining, x11). C: Higher-magnification photomicrograph of a bone bridge from group III, showing extensive loss of bone and replacement by cartilage (C), marked by the dashed line at the corner of the surface area (┐) (Giemsa staining, x60). Inset shows higher magnification of the area with bone loss and replacement by cartilage (C) (Giemsa staining, x150). D: Adjacent section to that shown in C after staining for TRAP activity. Dashed lines indicate borders between nonvital (NV) bone lacking cells positive for TRAP activity in bone and lacunae (∇), and vital (V) bone showing cells positive for TRAP activity in bone and lacunae (▲) and cartilage (C). Areas of necrosis and areas of cartilage are larger toward the corner (┐) of the surface area of the bone bridge (TRAP staining, x60). E: Photomicrograph of the middle of the rectangular surface area of a bone bridge in group IV (particles only), showing a fibrous tissue layer (FL). Toward the center of the bridge, there is bone loss with replacement by fibrous tissue (F). Particles (P) are surrounded by fibrous tissue. The remainder of bone is vital (V) (Giemsa staining, x55). F: Higher magnification of bone bridge in specimen from group IV (particles only) showing particles in clusters (∇), as individual entities, or intracellularly (▲). Giant cells are not seen (Giemsa staining, x250). G: Photomicrograph of bone bridge in group-IV specimen showing normal

TRAP activity in the bone bridge as well as in bone lacunae (BL), indicating vital bone (V). Inset shows a higher magnification of the area at P, with lacunae filled with particles and surrounded by TRAP-positive cells (∇). (TRAP activity staining, x55; inset, x200). H: Photomicrograph of bone bridge in specimen from group V (combined compression and particles), with the corner (†) of the surface area indicated. Areas of bone loss extend centrally into the bridge with replacement by fibrous tissue (F) and cartilage (C). Particles (P) are seen. The inset shows a higher magnification of this area, which demonstrates particles in clusters (∇), as individual entities, or intracellularly (▲) (Giemsa staining, x55; inset, x200). I: Photomicrograph of a specimen from group V (combined compression and particles), showing lacunae filled with clusters of particles (∇), surrounded by TRAP-positive activity (▲) (Giemsa staining, x250)

In group IV, in which high-density polyethylene particles had been introduced, fibrous tissue was not only present in the 100-µm-thick space on both sides of the bone bridges but it also replaced bone more centrally in the bone bridges. Fibrous tissue was more or less evenly distributed along the edges of the bone bridges, and it contained particles. Cartilage was sparsely present (Fig. 2, E). The presence of high-density polyethylene particles was confirmed by their typical birefringent appearance. Particles were present as individual entities, in clusters or intracellularly. Giant cells were not observed (Fig. 2, F). Qualitatively, the appearance of the bone tissue was normal, and TRAP activity and numbers of osteoclasts were present in amounts similar to that observed in the bone tissue in group I (the interface controls) and group II (controls). TRAP activity around lacunae filled with high-density polyethylene particles was not qualitatively increased compared with group I and group II. Necrotic areas of bone lacking TRAP activity were hardly present (Fig. 2, G).

After the combined administration of compression and high-density polyethylene particles in group V, both cartilage and fibrous tissue were observed to have formed in the bone bridge. High-density polyethylene particles were present in a similar way as in group IV (particles only). Giant cells again were not present (Fig. 2, H). Large areas of the bone bridges lacked TRAP activity, indicating necrosis. In these areas, we never observed particles. When lacunae were filled with particles, cells surrounding lacunae showed normal TRAP activity (Fig. 2, I).

Histomorphometry

As noted above, membrane compression in the absence of particles in group III (compression only) caused bone necrosis and loss of bone with replacement by cartilage. The areas of necrosis and cartilage were a median of 14% and 3%, respectively, of the surface area of the bone bridges (Table II, Fig. 3). The addition of high-density polyethylene particles without membrane compression in group IV (particles only) led to loss of bone with replacement by fibrous tissue. Fibrous tissue was found on a median of 6% of the surface area of the bone bridges. The combined application of compression and particles in group V induced necrosis, loss of bone with replacement by cartilage, and formation of fibrous tissue with median surface areas of 15%, 3% and 4%, respectively. Compared with groups I and II (the controls), these changes were significant ($p < 0.05$). When we compared the effect of the combined treatment with the effect of membrane compression alone or high-density polyethylene particles alone on the formation of necrosis, cartilage, or fibrous tissue, the changes were not significant.

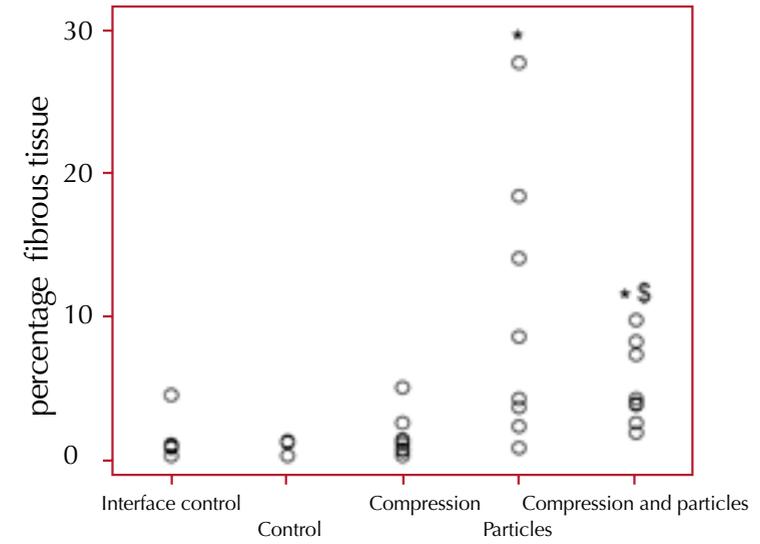
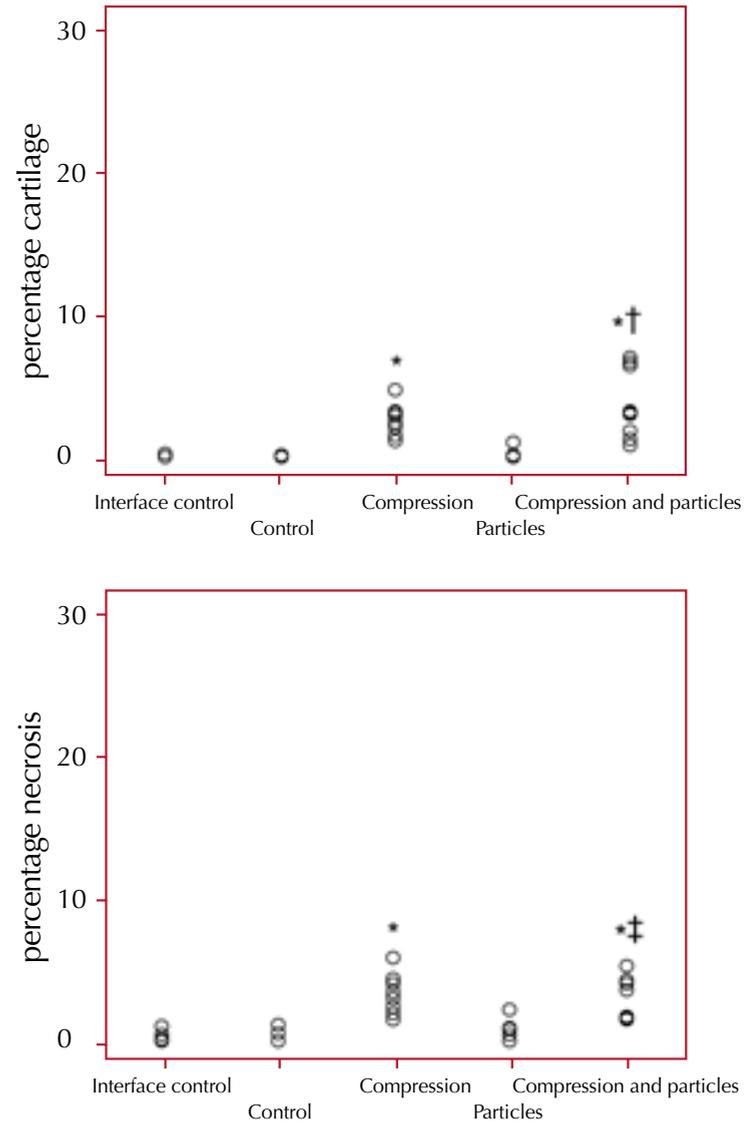


Fig. 3. Quantitative data on all individual animals with regard to the effect of mechanical compression of a fibrous tissue interface, introduction of high-density polyethylene particles, and a combination of the two on vital bone bridges in rabbits. The values are given as the percentages of the surface area of the bone bridges that were cartilage, necrosis, and fibrous tissue. *The difference between the treatment groups and groups I and II (the controls) with respect to cartilage, necrosis, and fibrous tissue was significant ($p < 0.05$). †The difference between group III (compression) and group V (compression and particles) with respect to cartilage was not significant ($p = 0.40$). ‡The difference between group III (compression) and group V (compression and particles) with respect to necrosis was not significant ($p = 0.76$). §The difference between group IV (particles) and group V (compression and particles) with respect to fibrous tissue was not significant ($p = 0.34$).

Discussion

Our model is intended to resemble the clinical situation of a prosthesis surrounded by a thin fibrous-tissue membrane producing micromovements upon weight-bearing, thereby compressing the fibrous tissue membrane. When components undergo early migration, the bone-prosthesis interface is unstable and interposition of fibrous tissue must be present. Successive component micromovements with compression of this fibrous tissue interface may generate a fluid pressure or fluid flow, leading to necrosis and subsequent loss of underlying bone. This may further impair prosthesis fixation and may signify the onset of clinical loosening. This proposed pathological mechanism corresponds with radiostereometric analysis studies that have shown that early migrating prostheses have a higher prevalence of loosening^{22,23}.

Fluid pressure leading to loss of bone has been hypothesized by Landells as early as in 1953³⁰. Indeed, recent animal experiments have shown that fluid pressure, either exogenously applied or through compression of a fibrous membrane, induces bone resorption^{17-21,31} and necrosis^{17,19,20}. On the basis of in vitro studies⁵⁻⁷, high-density polyethylene particles and polymethylmethacrylate particles have been shown to induce osteoclastic bone resorption by mediating inflammatory reactions. However, some in vivo studies have described a decrease in bone formation due to the presence of particles, without an increase in resorption^{10,11} or even loss of bone²¹. Therefore, it is still not clear whether and which particles cause loss of bone and, if so, whether this is through increased resorption by increased osteoclast activity or through decreased bone formation by inhibition of osteoblasts, or both.

In the present in vivo study in rabbits with a vital-bone prosthesis interface, we studied the effect of compression of a fibrous tissue interface, with or without high-density polyethylene particles, on bone and whether the combination of both stimuli has a synergistic effect on bone loss. High-density polyethylene particles were used because acetabular components of almost all total hip replacements, cemented and uncemented alike, are made of polyethylene and therefore seem to be the predominant particle type involved in the loosening process.

In all specimens of group I (the interface controls that had five weeks for healing after the first operation followed by two weeks without compression or particles), vital bone and a fibrous membrane of 100 µm on both sides of the bone bridge were present. In specimens in group II (the controls that had five weeks for healing followed by four weeks without compression or particles), the fibrous interfaces were

replaced by thin lamellae of new bone containing osteocytes and osteoid, thereby proving the vitality of the bone bridges and the tendency for bone formation under stable circumstances.

Compression of the fibrous membrane interposed between the implant and vital bone, by movement of the “dynamic” cylinder, led to areas of necrotic bone in the bone bridges with lacunae, indicating early stages of necrosis^{32,33}. This fibrous membrane consisted of cells and proteins and interstitial body fluid with physiologic properties. If it is assumed that the behaviour of such a fibrous membrane is biphasic, compression led to a combination of stress in the solid matrix and high fluid pressure in the fluid compartment^{24,25}.

Necrotic areas were situated underneath the fibrous tissue membranes at all four corners of the rectangular surface areas of the bone bridges, whereas bone tissue in the middle of the bone bridges was not altered. Since the amplitude of the movement of the cylinder was at its maximum at the corners of the rectangular surface areas, membrane compression and the resulting fluid pressure or fluid flow was likely maximum in these areas. This may explain why the greatest changes to the underlying bone occurred at the corners.

Van der Vis et al.¹⁹, using a similar model, compressed a fibrous membrane interposed between the implant and vital rabbit bone with the same amount of pressure and for the same duration and found necrosis as well. They discussed the possibility that pressurization of interstitial fluid leads to lethal disruption of the canalicular processes of osteocytes. Moreover, induction of an interstitial fluid flow may affect the interstitial balance in the extracellular matrix of bone with subsequent osteocyte death³⁴.

In the present experiment, newly formed bone always sealed off the implant and fibrous interface area from the medullary space; thus, we assume that this was a closed system. Furthermore, the implant we used was similar to the implant used by Van der Vis et al.^{17,18}, which has been shown to be watertight. Although our model can be considered as a closed system, reduction of the closed volume cannot be determined. We were thus unable to measure the pressure applied. We propose that by rotating the “dynamic” cylinder, the tissue fluid shifts lead to fluid flow as well as to a temporary local increase in fluid pressure. Therefore, the observed effects after membrane compression are likely to be due, at least in part, to the effects of fluid

flow or fluid pressure, although the exact volume ratio between the solid matrix and fluid compartment in this membrane is not known. The advantage of the current model over the model of Van der Vis et al.^{17,18} is that the compression of the fibrous interface better resembles the clinical situation (compression of a periprosthetic membrane during weight-bearing).

Compression alone led to cartilage formation. This cartilage formation was observed mainly at the four corners of the surface areas. Mesenchymal cells can differentiate into cartilage or fibrocartilage cells when hydrostatic pressure or hypoxia are applied³⁵. In vitro, chondrocytes produce matrix when hydrostatic pressures of 3 MPa are applied³⁶. In an experiment with a rat bone chamber with application of hydrostatic stress of 2 MPa, chondrocytes were formed, usually in combination with necrosis³⁷. Interestingly, necrosis was absent when chondrocytes had formed a continuous layer in between the site where the pressure had originated and the underlying bone³⁷. Those authors hypothesized that cartilage formation was a protective response of bone to fluid flow and/or fluid pressure and prevented necrosis. According to this hypothesis, the finding of necrosis in the compressed specimens in our experiment was to be expected because in none of these specimens was cartilage generated as a continuous layer: thus, it could not act as a protective barrier.

The presence of high-density polyethylene particles led to bone loss and replacement by fibrous tissue with no necrosis or cartilage. In other in vivo studies, high-density polyethylene particles led to loss of bone because of both decreased formation and increased resorption of bone³⁸, or because of increased resorption of bone only⁹, and bone resorption was associated with increased numbers of osteoclasts^{9,38}. In our experiment, bone loss in the presence of high-density polyethylene particles was not associated with an increase in numbers of osteoclasts. However, loss of bone can be the result of increased functional activity of osteoclasts without an increased number of cells³⁹. In addition, particles can suppress the function of osteoblasts⁴⁰ and inhibit proliferation and differentiation⁴¹ of osteoblasts. Therefore, loss of bone could have been caused by increased osteoclastic activity or by decreased osteoblastic activity, or both. However, fibroblasts exposed to particles respond with proliferation, possibly explaining the abundance of fibroblasts and the subsequent formation of fibrous tissue after particles were added in our experiment.

Polymethylmethacrylate particles are more potent in causing bone resorption than high-density polyethylene, at least in vitro⁴². However, Skoglund and Aspenberg²¹

found formation of bone after applying polymethylmethacrylate particles to a rat bone surface. They hypothesized that particles had been inactivated by opsonization. Another explanation may be that the particle size in that study ranged between 5-10 μm , which is at the upper limit for macrophage resorption²⁶, whereas the particles that we used were smaller (mean size, 4.8 μm), causing a stronger cellular response⁴³.

The combination of membrane compression and high-density polyethylene particles did not lead to a significant increase in the amounts of cartilage, necrosis, and fibrous tissue compared with the effect of one stimulus only. This indicates that compression of the fibrous membrane leads to necrosis and cartilage but not fibrous tissue formation, and treatment with high-density polyethylene particles leads to the formation of fibrous tissue but not to necrosis or cartilage. In other words, the different stimuli induced different effects on bone, and therefore a synergistic effect between both compression and high-density polyethylene particles was not observed.

Skoglund and Aspenberg²¹ found bone resorption after combined compression of a fibrous membrane with the administration of polymethylmethacrylate particles, but resorption was not greater than when pressure alone was applied. A synergism between pressure and particles on bone degradation could not be concluded. Particle release is a process that takes time³. By adding a surplus of particles, we attempted to simulate a situation that is present only after a total hip replacement has been in situ for long period. Several retrieval studies have shown results that are similar to our findings: interface tissue retrieved from clinically stable but migrating prostheses showed extensive cartilage formation in the presence of a fibrous tissue interface membrane^{44,45}. Necrosis has been observed in large amounts in fibrous membranes from total hip replacements retrieved because of aseptic loosening⁴⁶⁻⁴⁸, as well as in a series of retrieved interfaces that were being analyzed in our department at the time of writing (unpublished data).

The results of the present study suggest that compression of a periprosthetic fibrous interface, which induces fluid pressure and/or fluid flow, can be an important cause of early loosening of total hip replacements. Our results also suggest that polyethylene particles are involved in the loosening process as well, but probably only in later stages, when substantial amounts of these particles have been formed in the joint space³. The formation of cartilage may be a protective response of bone to the necrotic effect of fluid pressure and/or fluid flow, but further studies are needed to clarify this observation.

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