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The effects of micromotion and particulate materials on tissue differentiation

Bone chamber studies in rabbits

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Abstract

Motion at the interface between bone and implants for joint replacement may interfere with osseointegration and prosthesis stabilization. Particulate materials may cause foreign body and chronic inflammatory reactions resulting in bone resorption (osteolysis). The micromotion chamber (MC) and the bone harvest chamber (BHC) were implanted in the rabbit tibia, and the effects of micromotion and phagocytosable particulate materials on tissue formation within the chamber were assessed by studying bone ingrowth into a 1-mm pore.

Using the MC, one short daily episode of motion (20 cycles/day, 0.5 mm amplitude) for three weeks decreased the amount of bone ingrowth. Using a different pore configuration, the same parameters of motion increased bone ingrowth. Increasing the amplitude of motion (from 0.5 to 0.75 mm), or the number of daily motion periods (from one to two per day) then decreased bone ingrowth. These studies suggest the existence of a window of externally applied strain: a small stimulus may facilitate and a large stimulus may discourage bone formation within the chamber. Cessation of a given set of motion parameters (producing primarily fibrous tissue) for an additional three weeks was accompanied by tissue differentiation into bone.

Using the BHC, small, phagocytosable particles of bone cement, high density polyethylene and cobalt chrome alloy, at a concentration of 1.0×10^8 particles /mL, caused a foreign body reaction and inhibited the ingrowth of bone. Particles of titanium alloy had no effect on net bone formation. In studies using normal and immunodeficient rats, T lymphocytes were not a prerequisite for macrophages to phagocytose polyethylene particles.

In the clinical situation, micromotion and particulate debris may be synergistic in producing prosthetic loosening. If an implant does not undergo osseointegration due to excessive micromotion, the fibrous tissue interface may provide a conduit for the subsequent migration of particles around the implant.

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List of papers

This thesis is based on the following papers, referred to in the text by their Roman numerals:

I Aspenberg P, Goodman S, Toksvig-Larsen S, Ryd L, Albrektsson T. Short periods of micromotion inhibit bone ingrowth. An experiment using titanium implants in rabbits. Acta Orthop Scand 1992; 63 (2): 141–145.

II Goodman S, Song J, Doshi A, Aspenberg P. Cessation of micromotion facilitates bone formation in the micromotion chamber implanted in the rabbit tibia. Biomaterials 1994. In press

III Goodman S, Toksvig-Larsen S, Aspenberg P. Ingrowth of bone into pores in titanium chambers implanted in rabbits: Effects of pore cross-sectional shape in the presence of dynamic shear. Journal of Biomedical Materials Research 1993; 27: 247–253.

IV Goodman S, Aspenberg P. Effect of amplitude of micromotion on bone ingrowth into titanium chambers implanted in the rabbit tibia. Biomaterials 1992; 13 (13): 944–948.

V Goodman S, Wang J-S, Doshi A, Aspenberg P. Difference in bone ingrowth after one versus two daily episodes of micromotion: Experiments with titanium chambers in rabbits. J Biomedical Materials Research 1993; 27 (11): 1419–1424.

VI Goodman S, Aspenberg P, Wang J-S, Regula D, Emmanual D, Lidgren L. Cement particles inhibit bone ingrowth into titanium chambers implanted in the rabbit tibia. Acta Orthop Scand 1993; 64 (6): 627–633.

VII Goodman S, Aspenberg P, Song Y, Doshi A, Regula D, Lidgren L. The effects of particulate high density polyethylene and titanium alloy on tissue ingrowth into the bone harvest chamber in rabbits. Submitted for publication.

VIII Goodman S, Aspenberg P, Song Y, Doshi A, Regula D, Lidgren L. The effects of particulate cobalt chrome alloy and high density polyethylene on tissue ingrowth into the bone harvest chamber in rabbits. Submitted for publication.

IX Goodman S, Aspenberg P, Song Y, Doshi A, Regula D, Lidgren L. The effects of intermittent micromotion versus polymer particles on tissue ingrowth: experiment using the micromotion chamber implanted in rabbits. J Applied Biomaterials 1994. In press.

X Goodman S, Wang J-S, Regula D, Aspenberg P. T-lymphocytes are not necessary for particulate polyethylene-induced macrophage recruitment in the rat tibia. Acta Orthop Scand 1994; 65 (2): 157–60.

Definitions

Biomaterial: A material, whether degradable or not, that is placed within the body for a specific purpose.

Implant: Biomaterial placed within bone.

Arthroplasty: A surgical procedure on a joint in which part or all of the joint is resected with or without being replaced.

Prosthesis (joint endoprosthesis): An implant which replaces the structure and function of an excised bone or joint.

Interface: The junction between two different materials, or between a material and tissue.

Osseointegration: A process in which parts of an implant are in contact with bone without any intervening fibrous tissue at the light microscopic level, such that the mechanical loads are transmitted between implant and bone directly.

Micromotion: Small movements between a prosthesis (whether cemented or noncemented) and the surrounding bone, that are not detectable with conventional radiographic methods.

Migration: Gradual movement of a prosthesis over time from its original position.

Loosening: A process at the bone-implant interface by which a prosthesis does not become osseointegrated.

Radiographic loosening: A prosthesis that has migrated, or has a circumferential, 2-mm radiolucent zone.

Clinical loosening: A situation in which a patient complains of one or more of pain, shortening, deformity, and decreased function of a joint containing a prosthesis, that is radiographically loose.

Loosening membrane: Soft tissue found at the bone-implant interface of a nonosseointegrated prosthesis.

Osteolysis: Destruction of bone surrounding a prosthesis.

Wear: The erosion of one or more materials that are in contact and moving relative to eachother.

Introduction

There are differing opinions in the literature concerning the etiology and pathogenesis of prosthetic loosening.

Some groups have underlined the importance of biological factors at the bone-implant interface during the healing phase after the surgical trauma (Slooff 1971, Willert et al. 1974, Feith 1985, Rhinelander et al. 1979, Radin et al. 1982, Sund and Rosenqvist 1983). Charnley (1976,1989) demonstrated that a 0.5-mm wide area of necrotic bone and other tissue surrounded cemented hip prostheses several weeks after implantation. When this layer is remodeled, the initial stability of the prosthesis may be compromised and motion at the bone-implant interface may occur. Excessive motion may jeopardize osseointegration. According to this hypothesis, the ultimate fate of a prosthesis is defined wihin a short period of time postoperatively. Studies by several authors using roentgen stereophotogrammetric analysis (RSA) have corroborated this hypothesis. Using RSA, the amount of migration of prosthetic components at four months has been predictive of subsequent clinical outcome (Mjöberg 1986, 1991, Ryd 1986, 1990).

Other groups have implicated an important role for particulate materials within the bone-implant interface membrane of loose prostheses. Particulate materials have been associated with a foreign body and chronic inflammatory reaction (Bullough 1973, Heilmann et al. 1975, Vernon-Roberts and Freeman 1976, Willert and Semlitsch 1977, Mirra et al. 1976, 1982, Goodman et al. 1988 a,b); this may undermine the bone supporting the prosthesis (Goldring et al. 1982, 1986, Ohlin et al. 1990). In vitro studies have shown that when macrophages ingest particles, they become activated and produce factors known to stimulate the resorption of bone (Baggliolini et al. 1982, Williams et al. 1984, Herman et al. 1989, Murray and Rushton 1990). Supernatants from tissue cultures of the loosening membrane produce factors capable of inducing resorption in bone explants (Goldring et al. 1982, 1986, Ohlin et al. 1990).

The two explanations for loosening, namely micromotion and the response to particulate materials may be complementary. Some confusion regarding the cause of prosthetic loosening appears to stem from the lack of a rigorous definition of the term loosening. Because of a difference in the elasticity of bone and a metallic or plastic implant, a small amount of motion occurs at the bone-implant interface with the application of a given load (Volz et al. 1988, Yang et al. 1990). The strictest definition of loosening would be the presence of motion beyond that which normally occurs because of this difference in elasticity. If an intervening fibrous tissue layer exists at the entire bone-implant interface, increased micromotion or continuous migration may be detected by modalities such as RSA (Mjöberg 1986, 1991, Ryd 1986, 1990). Implants that have undergone osseointegration are in contact with bone without any intervening tissue at the microscopic level in some regions, such that the mechanical loads are transmitted directly from implant to bone. Only these implants can be regarded as truly "non-loose".

A prosthesis surrounded by a fibrous zone which is so extensive that it can be seen on conventional radiographs has been used as a definition of loosening (Charnley 1979). Symptoms such as pain with weight-bearing or torsional movements are part of a clinical entity, which is an even wider definition of prosthetic loosening (clinical loosening). However, radiographic findings and clinical symptoms often appear late in the loosening process (Mjöberg 1986, 1991, Ryd 1986, 1990). One can appreciate the confusion often generated when using the word loosening, and for the purpose of this thesis, the strictest of definition is used.

Both micromotion and particulate materials may thus play important roles in prosthetic loosening. If excessive micromotion is present during the healing period after prosthetic implantation, osseointegration of the implant will not occur (Pilliar et al. 1986, Engh et al. 1987, 1990, Søballe et al. 1992, 1993). A fibrous tissue layer will form at the bone-implant interface. This fibrous tissue layer may function as a conduit for migration of particles generated at the various interfaces. These particles may elicit an influx of foreign body and chronic inflammatory cells that may initiate bone resorption.

How much micromotion is compatible with osseointegration of a prosthesis? In related biological processes such as fracture healing, load and motion can enhance bone formation (Wolf et al. 1981, Goodship and Kenwright 1985, Rubin and Lanyon 1985). Could the mechanical environment at the bone-implant interface be manipulated to facilitate the process of bone ingrowth? Pilliar et al. (1986) has shown that repetitive micromotion of up to 28 μm in amplitude can still permit ingrowth of bone into porous coated implants. What are the effects of a single, daily episode of externally applied micromotion on bone ingrowth? To study

the relationship between micromotion and bone ingrowth, we have designed the micromotion chamber (MC) for implantation in the proximal tibia of the rabbit. The MC allows periodic harvesting of the tissue that has grown into a 1-mm pore. The model is unique in that the investigators can manually deliver specific, discrete, daily periods of motion of a predetermined amplitude and frequency to the ingrowing tissue.

Many properties, such as the size, shape, topography, surface charge, surface chemistry etc. are important in determining the tissue reaction to implanted particles (Nagura et al. 1977, Besterman et al. 1983, Kawagushi et al. 1986, Tabata et al. 1988, Barth et al. 1991). However, from a practical point of view, a good starting point is to delineate which particulate materials of a given size are most deleterious to bone ingrowth. Materials used for prostheses have produced little or no inflammatory reactions in bulk form. Few studies have examined the effects of different particulate materials on bone ingrowth using an in vivo model. To this end, we have employed the Bone Harvest Chamber, a titanium device that is implanted in the rabbit tibia and allows repeated harvests of the tissue that grows into a pore in the chamber (Albrektsson et al. 1984). This model focuses on net bone formation in the presence of phagocytosable particles of different materials. The model is especially relevant to the ingrowth of bone into porous coated implants. As bone is a dynamic organ, constantly undergoing both formation and resorption, studies on particulate materials in this model may also have broader implications for the remodeling process of the prosthetic bed.

LITERATURE REVIEW

The effects of mechanical stimulation on the differentiation of bone

Recent studies have shown that the mechanical environment is important in the differentiation and development of mesenchymal tissue. Understanding this interaction may improve the outcome for various musculoskeletal conditions, by the application of well-defined, exogenous loads. In the following, the literature is examined, emphasizing two areas: the healing of fractures, and bone ingrowth into porous coated prostheses.

General studies on load and bone remodeling

Load in intact bones

The deformation of bone during loading has been quantitated in vivo with the use of strain gauges. Despite vast differences in the size and style of locomotion of a wide range of animal species (including humans), the peak strains in loadbearing bones are remarkably similar, possibly indicating similar strain thresholds for bone remodeling (Lanyon and Smith 1970, Lanyon et al. 1975, Rubin and Lanyon 1984).

The mechanical environment and bone remodeling

Wolff (1892) first postulated that the mechanical environment maintains bone mass and determines bone remodeling. This hypothesis has been tested using a unique animal model (Rubin and Lanyon 1984, Lanyon 1987, Rubin et al. 1990). The diaphysis of the ulna of skeletally mature roosters was functionally isolated by surgical means, by excising the articular segments of the ulna and applying an external fixation device. When load bearing was removed for six weeks, the isolated ulnar segment demonstrated a reduction of bone mass and intracortical porosis and resorption. When the isolated ulnar segment was subjected to physiological loading of 4 cycles per day of 0.010–0.012 strain at 0.5 Hz for six weeks, the disuse osteopenia was prevented. If the loading was increased to 36 cycles per day, bone accretion was seen. Thus, alterations in the mechanical environment were associated with dramatic changes in bone mass.

Using the ulna of mature male turkeys, Rubin and Lanyon (1985) developed a dose-response curve for peak strain magnitude and alteration in bone mass. Peak longitudinal strains below 0.001 (100 daily loading cycles of 1 Hz for 8 weeks) produced loss of bone mass, whereas those above 0.001 produced marked periosteal and endosteal bone formation compared to intact bones. Skerry et al. (1986), using the same model

and a loading pattern of 360 sinusoidal cycles from 0 to 600 N (producing a longitudinal strain of 0.002) at a frequency of 1 Hz showed that a short period of dynamic loading could influence optical polarization of the bone matrix, presumably by changing the orientation of the proteoglycan molecules in bone. This phenomenon was observed immediately after the application of the load and reverted to normal 48 hours after cessation of the load. It was proposed that this phenomenon provided a mechanism whereby the bone's recent dynamic strain history could be recorded, enabling the bone to adapt to changes in load in the functional environment.

What are the cellular mechanisms by which load affects tissue remodeling? In vitro studies have demonstrated the ability of the osteoblast to respond to strain by alterations in the production of PGE2, cAMP, cGMP, DNA, and other substances (Somjen et al. 1980, Burger et al. 1988). Cyclic but not static loading of osteoblasts in tissue culture stimulated cellular proliferation but suppressed the synthesis of proteoglycans, collagen and noncollagenous protein (Strafford et al. 1989). Other studies have suggested that the extracellular matrix may provide a three dimensional framework that conveys or transforms the mechanical stimulus for the osteoblast to interpret (Rodam et al. 1975). Recent studies have also underlined the importance of electrical fields in the maintenance, remodeling and repair of bones (Lavine and Grodzinsky 1987, McLeod and Rubin 1992).

Finite element studies and bone remodeling

Theoretical investigations using finite element modeling have demonstrated that the mechanical loading history can direct the process of endochondral ossification and the eventual architectural configuration of bones (Carter 1987, Carter et al. 1988, 1989, 1991, Wong and Carter 1990). This determination apparently begins at a very early stage in skeletal development according to mathematical "rules of construction", which hypothetically provides a mechanism for the development, differentiation and repair of tissues of the musculoskeletal system and the adaptation of the organism to alterations in the biomechanical milieu.

The effects of load on fracture healing

Several research groups have tried to influence fracture healing by the application of mechanical load. Early loading of the fractured rat femur was associated with earlier, more pronounced fracture healing radiographically, histologically and mechanically (Sarmiento et al. 1977). This concept has been applied to humans with encouraging results (Sarmiento 1972).

Chao's group studied the effect of initial fracture stability on fracture healing using standardized, bilateral tibial osteotomies in mature dogs. After 120 days, a more rigid method of fixation using a plate and screws facilitated earlier healing and remodeling of the fracture compared to external fixation using half pins (Lewellan et al. 1984). When comparing different methods of external fixation, a less rigid construct was associated with a prolonged time for repair and remodeling (Wu et al. 1984, Williams et al. 1987). These studies emphasized that initial stability of the fracture fragments promotes earlier fracture healing. Compression of the fracture fragments, whether static (80 Newtons) or dynamic (telescoping with gait) did not alter the progression of healing (Hart et al. 1985, Aro et al. 1990). The apparent discrepancy in the conclusions reached by Sarmiento's group versus Chao's group with respect to early weightbearing may be due the choice of model (rat with a femur fracture treated with intramedullary fixation and weightbearing versus dog with a tibial fracture treated with external fixation). Specifically, intramedullary fixation in the former group may have provided more initial stability and thus a more favorable milieu for fracture healing with weightbearing compared to external fixation in the latter study.

Wolf et al. (1981) hypothesized that the intermittent cyclic strain produced by an externally applied, *well controlled* cyclic load was a more important stimulus to fracture healing than simple static compression. Rabbits were subjected to bilateral tibial osteotomies fixed with external fixation. By 4–6 weeks, the tibia undergoing controlled cyclic loading (40 N at a frequency of 55 times per minute, three hours every morning and evening) demonstrated higher torque and energy absorption to failure and lower stiffness compared to the contralateral tibia in which 80 N of constant compression was applied. In the early stages of fracture healing, it would appear that stability of the fracture fragments hastens the repair process. Thereafter, controlled cyclic loading may accelerate remodeling of the callus.

Goodship and Kenwright (1985, 1990) also found that fracture healing was responsive to an imposed, controlled, mechanical environment. Osteotomized sheep tibiae fixed with external fixation and undergoing controlled axial loading (500 cycles of 360 N delivered axially at 0.5 Hz for 17 minutes daily) demonstrated more external callus, increased fracture stiffness and more advanced fracture healing histologically compared to a group in which the osteotomy site was distracted 3 mm. A larger initial gap between the bone ends (0.5 versus 2.0 mm) proved to be detrimental to the healing of the osteotomy, even with the application

of micromovement (Kenwright and Goodship 1990). Healing was mechanically and histologically more sound in osteotomies subjected to 200 N compared to 1000 N of intermittent micromotion (delivered over 17 minutes at 0.5 Hz for 12 weeks), implying the existence of an upper limit to the positive effects of externally applied load on fracture healing.

Controlled cyclic load and fracture healing in humans
Kenwright et al. (1991) have utilized an imposed mechanical load in the treatment of tibial fractures stabilized with external fixation. A group of patients subjected to cyclical axial displacement of 1.0 mm at 0.5 Hz for 20 - 30 minutes per day using a pneumatic pump attached to the external fixator healed their fracture in a shorter time clinically, radiographically and biomechanically compared to a second group treated with external fixation alone

Summary of the effects of load on fracture healing
It would appear that, after a period of protected loading, fractures stabilized with external fixation demonstrate an increased rate of healing with the application of controlled, cyclic loading. Variables such as the size of the initial gap, the stability of the bone-fixator construct, and how the motion is applied appear to be important.

Micromotion and bone ingrowth into porous coated prostheses

Noncemented, press-fit prostheses with/without porous coating, surface texturing etc. have been employed as a method of prosthetic stabilization (Galante et al. 1971, Spector 1987). Porous coated implants depend on bone ingrowth into small pores. Two systems of porous coating have evolved, the microporous system (pore size approximately 100–500 μm) and the macroporous or madreporic system (pore size of 1–2 mm). In the microporous system, the pore size appears to be crucial to the degree of bone ingrowth (Bobyn et al. 1980).

Several groups have measured the amount of micromotion at the bone implant interface in vivo and in vitro. Yang et al. (1990), using fresh frozen canine tibiae implanted with a tibial prosthesis, showed that tangential displacement ranged from approximately 40 μm during application of 1 times body weight, to 100 μm with 4 times body weight. Volz et al. (1988), using four uncemented tibial component designs, found that after 300,000 cycles of loading from 5–115 kg, motion at the bone implant interface ranged from less than 100 μm to greater than 500 μm. In canine studies using a hip prosthesis, Vanderby et al. (1989) found that torsional (craniocaudal) loads resulted in motion ranging from less than 50 μm for cemented components, to 300 μm (at 4 months of ingrowth) and 500 μm (immediately postoperatively) for porous coated prostheses. Zalenski et al. (1990), using a titanium fiber mesh porous coated hip prosthesis implanted into the canine femur measured 7–56 μm of rotational micromotion immediately after prosthetic implantation but only 0.6–25 μm of motion after 6 months. Anderson et al. (1990, 1991), using a madreporic, cobalt chrome canine hip prosthesis and axial loads of up to 300 N documented motion in the order of 65 μm or less postoperatively, and less than 27 μm after 2 years. The micromotion using comparable cemented prostheses was 7–18 μm.

The amount of bone ingrowth into porous coated prostheses in humans has been disappointingly small (Cook et al. 1988, Cook 1991). One possible reason is the initial instability secondary to muscle forces and intermittent loading (Cameron et al. 1973). However, some degree of micromotion is compatible with bone ingrowth. Pilliar et al. (1986) has shown in dogs that up to 28 μm of micromovement can still permit bone ingrowth to occur. Movement at the interface measuring 150 μm or greater promoted the formation of fibrous tissue. Similar results were reported by Burke et al. (1991). Hollis et al. (1992) implanted multiple porous coated titanium plugs transcortically into mature dogs. Using a special device, adjacent implants were rotated 25, 50, 100, or 200 μm twice per day for 10 minutes. With 25 μm of motion, bone grew consistently into the pores; little or no bone ingrowth was seen with 200 μm of rotational motion. With motion in the range of 50–100 μm, the amount of bone ingrowth was related to the magnitude of motion and the size of the pore. This study also showed that short periods of daily micromotion can inhibit bone ingrowth.

The placement of an undersized screw within bone is somewhat analagous to the process of bone ingrowth into a porous coated prosthesis in that the bone in the threads of the screw must undergo a process of repair and remodeling in order to maintain adequate stability of the construct. Schatzker et al. (1975) placed tightly fitting and undersized screws into loaded, and minimally loaded long bones in dogs. Screws located in a stable, minimally loaded environment, whether undersized or not were surrounded by new bone at six weeks. However, undersized screws subjected to intermittent loading during gait were surrounded by a synovial-like lining, fibrous tissue and osteoclastic resorption of the surrounding bone. This study points to the importance of the biomechanical environment in stabilizing an implant with a poor initial fit within bone and that osteolysis around an implant can be mechanically induced without the presence of particulate materials.

Hydroxyapatite (HA) coatings have been used to improve the fixation of cementless components. Søballe and coworkers (1992) have shown that coating a porous titanium implant with HA improves bone ingrowth from 8 percent (uncoated) to 47 percent for stable implants after 4 weeks. When the implants were unstable (a dynamically loaded device produced 150 or 500 μm of axial translation during each gait cycle), HA coating improved pushout strength but not the amount of bone ingrowth (Søballe et al. 1992,1993). A thick fibrous membrane was noted around unstable titanium implants but a thinner fibrous/fibrocartilaginous membrane with a radiating rather than a random collagen pattern surrounded HA coated implants. When micromotion was discontinued between 4 and 16 weeks, the fibrous and fibrocartilaginous tissue around both types of implants were replaced by bone, indicating a capability of the tissue to remodel into another type of differentiated tissue of mesenchymal origin.

Prosthetic design and surgical technique have been shown to affect the degree of prosthetic micromotion and bone ingrowth. Evans et al. (1990) demonstrated a linear, inverse relationship between the degree in which a straight stemmed, collarless titanium alloy prosthesis filled the femoral canal, and the amount of micromotion. The distal, non-porous coated part of the femoral stem was shown by Jasty et al. (1993) to enhance initial stability to axial and rotational loads, but this portion of the stem was found to be superfluous shortly after implantation. Increasing the femoral component offset (which was shown to be an advantage in cemented femoral components), may be a possible disadvantage in cementless femoral hip components because of the resulting increase in micromotion during stair climbing (O'Connor et al. 1989). Under-reaming the femur distally by 0.5–1 mm was found to improve torsional stability of femoral components (Sugiyama et al. 1990).

Controlled cyclic load and bone ingrowth

Can mechanical strain enhance bone ingrowth into porous coated prostheses? Rubin and McLeod (1992) implanted 5 mm diameter porous coated titanium alloy rods into functionally isolated ulnae of skeletally mature male turkeys. A servo-hydraulic actuator provided controlled dynamic loading of the ulna at 1 or 20 Hz for 1000 cycles/day. Ingrowth of bone was enhanced by both loading frequencies, yielding means of 21 percent and 74 percent bone ingrowth with the lower and higher frequencies respectively, compared to 13 percent ingrowth without any load. Thus, bone stimulated by a controlled, frequency specific, low amplitude mechanical load may have an increased capacity for bone ingrowth.

Finite element analysis of bone ingrowth and remodeling

The general principles governing the remodeing of bone are applicable to the situation in which a prosthesis is implanted (Rubin and Lanyon 1984, 1985, Huiskes et al. 1987). Using the theories of adaptive bone remodeling, the Strain Energy Density has been noted to be the key parameter of a feedback mechanism determining the configuration and architectural appearance of bone with an implant (Huiskes et al. 1987). The rigidity of the implant and the bonding characteristics of the implant to bone are important determinants of the remodeling process.

Summary of the effects of load on bone ingrowth

To optimize bone ingrowth, porous coated prostheses should be implanted so as to obtain minimal initial micromotion and close apposition between the porous coating and the adjacent bone. The anatomy, design of the prosthesis, and surgical technique are crucial variables. Repetitive micromotion above 25–50 μm appears to inhibit bone ingrowth. Specific, controlled, exogenous, loads may stimulate the bone surrounding a porous coated implant.

Summary

Theoretical and experimental studies have shown that the differentiation and development of mesenchymal tissue is determined in part by the loads to which it is subjected. The possibility of modulating processes such as fracture healing and bone ingrowth into prosthetic implants by altering the biomechanical environment is intriguing. With respect to bone, it would appear that thresholds of mechanical strain exist which may facilitate or discourage the accretion of bone.

The effects of particulate materials

The properties of the loosening membrane and the importance of particulate debris will be reviewed to help elucidate the cellular basis of prosthetic loosening.

Histological properties of the membrane

In order to understand the pathophysiology of loosening and prosthetic osteolysis, we need information about the tissue surrounding successful prostheses. Autopsy studies have reported contradictory results. Charnley (1976, 1989) described the histological findings in 23 clinically successful human specimens harvested between one month and seven years after cemented total hip replacement. Several weeks after implantation, a tissue layer 0.5 μm adjacent to the cement demonstrated cellular damage (the borders of fat, haematopoietic and bone cells became unclear) presumably due to chemical, thermal and mechanical trauma. Several months to a year after surgery, a fibrous tissue layer was formed at the interface, which underwent metaplasia to fibrocartilage in areas subjected to "mechanical pressure." The necrotic bone adjacent to the cement was slowly replaced by new bone, with an intervening layer of fibrous tissue or fibrocartilage. The fibrocartilage sometimes underwent metaplasia to lamellar bone over many years. A foreign-body giant cell reaction was seen in areas that Charnley believed were subject to minimal load.

A recent autopsy study of 16 femora retrieved from asymptomatic patients who received a hip prosthesis two weeks to 17 years earlier showed trabecular bone intimately interdigitated with the cement (Maloney et al. 1989, Jasty et al. 1990, 1991). Fibrous tissue was reported to be rare at the cement-bone interface. The surrounding bone had remodeled, forming a secondary, circumferential, trabecular "neocortex" adjacent to the cement. The authors concluded that failure of fixation of cemented femoral components was initiated not at the cement-bone interface, but at the cement-prosthesis interface by separation or "debonding" and fracture in the cement mantle. When the cemented acetabular components from these autopsy cases were examined, the findings indicated that the progressive resorption of the bone supporting the prosthetic bed was the result of a foreign body response to particulate polyethylene (Schmalzried et al. 1992).

In a similar study of 14 successful, cemented hip prostheses harvested 2 weeks – 14 years after implantation, cemented components were surrounded by a fibrohistiocytic membrane, similar to that surrounding clinically and radiographically loose prostheses (Fornasier et al. 1991). Histiocytes and giant cells were observed surrounding and engulfing cement and polyethylene debris; chronic inflammatory cells were also noted in a background stroma of fibrous tissue. Fibrocartilage often covered thickened bony trabeculae. The density of histiocytes correlated with the time after implantation, the thickness of the membrane, and the density of small particles of polyethylene at the interface. The underlying bone demonstrated remodeling. This description of the interface suggested "loosening in evolution" (similar to the description by Schmalzried et al. 1992), rather than intimate bone-cement interdigitation (Maloney et al. 1989, Jasty et al. 1990, 1991).

Failed cemented prostheses are surrounded by a tan-colored, rubbery tissue composed of mono- and multinucleated histiocytes and chronic inflammatory cells in a fibrous stroma (Heilmann 1975, Mirra et al. 1976, Vernon-Roberts and Freeman 1976, Bullough 1977, Willert and Semlitsch 1977, Pizzoferrato 1979, Mirra et al. 1982, Bell et al. 1985, Johanson et al. 1987, Maguire et al. 1987, Pizzoferrato et al. 1988, Willert et al. 1990). Goldring et al. (1983, 1986) observed a thin, synovial-like lining layer, composed of large polygonal cells with eccentric nuclei at the surface of the membrane, adjacent to the cement layer. Beneath this layer was a loose fibrovascular stroma containing mono- and multi-nucleated histiocytic cells and wear particles. A third layer of more dense fibrous tissue abutted the surrounding bone. The synovial-like layer has not been identified in all clinically and radiographically loose specimens (Bell et al. 1985, Johanson et al. 1987, Goodman et al. 1989) and probably reflects the presence of motion at the bone-cement interface; a synovial-like layer has also been found at the interface of loose, overdrilled (as opposed to tight-fitting) screws implanted in bone (Schatzker et al. 1975).

Depending on the materials used in the prosthesis, small particles of bone cement, polyethylene and metallic debris are intermixed with this cellular reaction. The presence of polymethyl methacrylate (PMMA), which is dissolved during routine processing of the tissue specimens, can be surmised by the identification of round vacant cement "ghosts" and the presence of residual, undissolved particles of the radiographic contrast agent, approximately 1–100 μm in diameter. Particles of cement up to 10–30 μm can be found within macrophages and foreign body giant cells (Goodman et al. 1988a). Larger cement "lakes", several hundred micrometers in diameter within the membrane are usually lined by a fibrous tissue layer containing macrophages and giant cells.

The polyethylene debris within the membrane can be

identified histologically with the use of polarized light or oil red O staining (Goodman et al. 1988b, Campbell et al. 1992, Peters et al. 1992). The polyethylene debris varies from submicron particles to larger, thread-like shards 1 mm or more in length. Particles smaller than approximately 10 μm can be found within mononuclear histiocytic cells, whereas larger particles up to approximately 30 μm are found within multi-nucleated cells (Stinson 1964, Goodman et al. 1988b, Goodman et al 1990a). Larger particles which can not undergo phagocytosis are usually surrounded by multi-nucleated giant cells.

Black metallic particles are generally up to several micrometers in diameter and located both intra- and extra-cellularly (Rae 1975, 1981, 1986, Buchert et al. 1986, Agins et al. 1988, Howie and Vernon-Roberts 1988). When in small numbers, these particles are often intermixed within the fibrous tissue stroma, and within occasional scattered histiocytic cells (Goodman et al. 1990, 1992). In greater numbers, this debris is associated with a more extensive fibrohistiocytic reaction (Agins 1988). The tissues may have a black discoloration to gross examination.

In human studies, cement, polyethylene and metallic particles found within large numbers of foreign body cells have been associated with localized osteolysis (scalloping) or more widespread areas of lysis of the periprosthetic bone mantle (Jones and Hungerford 1987, Anthony et al. 1990, Maloney et al. 1990a, b, Schmalzried et al. 1992).

Clinically successful, cementless metallic prostheses are surrounded by a fibrous tissue layer or bone. Surprisingly, bone comprised less than 10 percent of the ingrowth area of porous coated implants that were revised for reasons other than aseptic loosening; fibrous tissue was more prominent (Cook et al. 1988, 1991). Dense connective tissue was found at the bone-metal interface of both successful and failed Moore-type uncemented prostheses (Kozinn 1986). Fibrocartilage was found in areas undergoing direct compressive load.

Histological studies performed in animals have confirmed that, in general, bulk forms of materials placed within bone or soft tissue evoke a fibrous tissue encapsulation (Goldring et al. 1986, Goodman et al. 1988a, b, 1990b). Some metals (for example commercially pure titanium) may undergo osseointegration (Sennerby 1991). Particulate materials stimulate a foreign body and chronic inflammatory reaction (Stinson et al. 1964, Goldring et al. 1986, Paiement et al. 1986, Goodman et al. 1988a, b, 1990a). In one study, particles of high density polyethylene (20–200 μm) were repeatedly injected into the rat knee joint, which contained a non-weightbearing cement plug placed in the intercondylar area of the distal femur. The plug became surrounded by a foreign body membrane containing particles of polyethylene and eroded the underlying bone (Howie et al. 1988). Thus, the histological picture evoked by polyethylene debris was similar to that of human autopsy studies summarized previously (Fornasier et al. 1991, Schmalzried et al. 1992). The particles used in this animal study were generally not of a phagocytoseable size which implies that cellular internalization of the particulates may not be necessary to initiate the events leading to the resorption of bone.

Biochemical properties of the membrane

The tissue at the bone-implant interface of clinically loose prostheses is very active metabolically. Many of the substances produced by this tissue modulate the inflammatory reaction, regulating different cellular interactions and the remodeling of bone.

High levels of prostaglandin E_2 (PGE_2) have been assayed from cultures of tissue harvested at revision for aseptic loosening (Goldring et al. 1983, 1986, Jasty et al 1984, Goodman et al. 1989, Ayers et al. 1989, Mather et al. 1989, Ohlin et al. 1990). Indeed, two studies have documented higher levels of PGE_2 from the membrane surrounding clinically loose components compared to successful components (Jasty et al. 1984, Goodman et al. 1989). This finding was recently corroborated using an animal model of a "loose" versus a "well-fixed" tibial hemiarthroplasty (Goodman et al. 1992). Furthermore, higher levels of PGE_2 were produced in culture of the tissue surrounding particulate versus bulk forms of bone cement implanted in the rabbit tibia (Goodman et al. 1990c). Thus it would appear that prosthetic loosening, the presence of particulate debris and PGE_2 production are related.

PGE_2 is an arachidonic acid derivative produced by phagocytic cells (polymorphonuclear leukocytes and macrophages) that is pro-inflammatory (Raisz and Martin 1984, Goetzl and Goldstein 1985, Zurier 1988). PGE_2 acts synergistically with other mediators to augment the intensity and duration of pain. PGE_2 has also been shown to stimulate the resorption of bone in vivo and in vitro (Klein and Raisz 1970, Raisz and Martin 1984, High 1988). These facts have suggested that PGE_2 may play an important role in the pain and the periprosthetic resorption of bone associated with loose prostheses. Nonsteroidal anti-inflammatory drugs, which inhibit the production of prostaglandins may have a role in mitigating the pain and progressive resorption of bone associated with prosthetic loosening (Goetzl and Goldstein 1985, Goodman et al. 1991).

Recent studies have implicated a possible role for

several cytokines including interleukin-1 (IL-1), interleukin-2 (IL-2) and interleukin-6 (IL-6), tumor necrosis factor (TNF), osteoclast activating factor (OAF) and transforming growth factor beta (TGFß) in the biological activities of the tissue at the bone-implant interface (Appel et al. 1988, Kim et al. 1988, Goodman et al. 1989, Mather et al. 1989, Chiba et al. 1992). Many of these factors are powerful inducers of bone resorption.

The supernatants from cultures of loosening membranes harvested from revision cases and the newly formed periprosthetic capsule were shown to induce bone resorption using the mouse calvarial assay (Ohlin et al. 1990). Part of the bone resorption was correlated with the production of PGE_2. Dorr et al. (1990) compared PGE_2, IL-1 and collagenase levels in tissue harvested from clinically loose with those from successful cemented and cementless prostheses. The authors could not find any biochemical factors that distinguished any particular subgroup. Neither could they show any effect of different materials used for the femoral component. One problem with biochemical studies using tissue specimens harvested at surgical revision is sampling error. Wide variations in the histological and biochemical results are seen depending on the location of the harvested specimen. Future studies must address this issue by processing multiple specimens from several different locations, and correlating the biochemical and histological results.

Biochemical data from several, short-term animal models that simulate prosthetic loosening demonstrated increased levels of PGE_2 and other inflammatory mediators in culture of the tissue surrounding clinically loose, cemented implants (Spector et al. 1990, Goodman et al. 1992b). A standardized model of prosthetic loosening using animals would be very useful, because it is impossible to control the myriad of patient, technical and prosthetic variables when one harvests a piece of tissue from humans at revision surgery. However, these short-term models, in which the prosthesis is grossly mechanically loose per primum, may not represent the clinical entity seen in humans.

Cell culture studies

In determining the biocompatibility of materials, cell culture studies are useful in delineating the response of a relatively uniform population of cells to well-characterized materials in different forms. However, it must be kept in mind that in vitro studies are performed in a closed system that is isolated from the influence of other organs.

Phagocytosis can result in the release of mediators of inflammation and bone resorption (Baggliolini et al. 1982, Williams et al. 1984). Exposure of cultures of polymorphonuclear leukocytes (PMN) to particles of polymethylmethacrylate (PMMA) 50–60 nm in diameter resulted in the release of the lysosomal contents and inhibition of PMN migration in a dose-dependent manner (Pappatheofanis and Barmada 1991). In another study, particles of PMMA 1–150 µm in diameter were cultured with peripheral blood monocytes. The culture supernatants demonstrated increased levels of PGE2, tumor necrosis factor and interleukin-1-like activity (using the thymocyte proliferation assay) and produced the resorption of bone using a ^{45}Ca-labeled bone assay in mice (Herman et al. 1989). In another study, Simplex polymer powder (Howmedica, Rutherford NJ, USA), approximately 1–100 µm in diameter stimulated the proliferation of adherent cells (monocytes/macrophages); the supernatants from the cell cultures with particulate cement demonstrated increased b-glucosaminidase activity (a lysosomal enzyme), but interleukin-1-like activity (using the thymocyte proliferation assay), and PGE_2 release were not stimulated (Davis et al. 1989, 1990). Macrophages harvested from the granulomas formed by the subcutaneous implantation of PMMA particles (50–200 µm) in mice produced low-grade surface and high-grade lacunar osteolysis on bone explants (Quinn et al. 1992). Thus PMMA particles can stimulate the release of factors that are involved in the resorption of bone.

Are macrophages activated equally by particles of different materials? Mouse peritoneal macrophages were exposed to different orthopedic materials in particulate form (Murray and Rushton 1990). Zymogen and latex particles were used as positive and negative controls respectively. Dose-response curves were calculated using a concentration of 10^6 cells/mL. For latex particles approximately 1 µm in size, a concentration of 10^8 particles/mL stimulated an increased release of PGE2 and ^{45}Ca using the neonatal mouse calvarial assay. Zymogen particles caused similar effects at a much lower concentration (10^6 particles/mL). An intermediate concentration was found for particles of PMMA or high density polyethylene. None of the four materials was toxic to the macrophages in the dosages used. Using cell cultures of P388D1 murine macrophages, PMMA but not polystyrene particles less than 1 µm in size were shown to inhibit the synthesis of DNA (at a concentration of 7×10^9 particles per 5×10^6 cells), and impair the ability of activated macrophages to kill mast cells (Horowitz et al. 1988).

To summarize, activated macrophages appear to play an important role in the pathogenesis of the bone resorption around orthopedic implants. Particulate materials with different properties stimulate macro-

phages. Some of the important properties include the size, shape, topography, surface charge, and surface chemistry of the particles (Nagura et al. 1977, Besterman et al. 1983, Kawagushi et al. 1986, Tabata et al. 1988, Barth et al. 1991). The particulate materials do not have to be of a phagocytosable size to activate macrophages or stimulate the resorption of bone (Howie et al. 1988, Quinn et al. 1992).

Immunological studies of the membrane

Immunohistochemistry and *in situ* hybridization (Myerson 1988, Nakmura 1990) have recently been used to assess the properties of membranes harvested at revision. Immunohistochemical studies use monoclonal antibodies to identify antigens that are specific for certain cell types. *In situ* hybridization identifies specific mRNA for proteins such as various interleukins and growth factors. In general, these factors are very short-lived and difficult to assay by other methods.

Immunological studies of the loosening membrane have been contradictory. In one study that examined the membrane from cementless loose acetabular components, particles of titanium and polyethylene were thought to cause the migration, adherence and phagocytosis of macrophages that were CD11b-positive but peroxidase-negative (Santavirta et al. 1991). However, the authors could not provide histochemical evidence of activation of the immune system (staining for interleukin-2 receptor-positive activated T cells and PCA-1 plasmablasts/plasma cells was negative). In another study, the histochemical staining of the tissues harvested from around failed titanium prostheses suggested the presence of a cell-mediated immune process (Lalor et al. 1990). Abundant macrophages and T lymphocytes but not B lymphocytes were present (Lalor et al. 1990, 1991, Lalor and Revell 1992). In a study comparing retrieved membranes from clinically loose with successful cemented and cementless arthroplasties, macrophages and T but not B lymphocytes were identified in nearly all specimens (Goodman et al. 1992c).

In a recent study of the tissues harvested from clinically and radiographically loose polyethylene cups, macrophages, fibroblasts and T lymphocytes were identified using immunoperoxidase staining (Jiranek et al. 1993). *In situ* hybridization studies noted the presence of interleukin-1-ß and platelet-derived growth factor-2 messenger RNA within macrophages; interleukin-1-ß protein was localized on the surface of macrophages and fibroblasts. These cytokines were postulated to be involved in bone resorption, fibrous proliferation and eventual prosthetic loosening.

In an ongoing retrieval study using immunohistochemistry and *in situ* hybridization, the membranes from clinically and radiographically loose cemented prostheses with osteolysis contained significantly greater numbers of macrophages, total T lymphocytes and cytotoxic T lymphocytes compared to loose cemented and cementless prostheses without osteolysis (Huie et al. 1993). These findings suggest a role for the cell-mediated immune response in the process of osteolysis. The *in situ* hybridization studies have demonstrated that TGF-ß and IL-6 appear to be associated with the process of osteolysis around cemented components. These factors are known to modulate the growth, proliferation and differentiation of cells of the immune and the monocyte/macrophage systems, and to mediate the remodeling of bone and other mesenchymal tissues (Goldring and Goldring 1990, Nathan 1991, Pfeilschifler et al. 1991, Vitetta and Paul 1991).

Summary

Histological studies of the tissue from failed, clinically and radiographically loose prostheses have emphasized the importance of the soft tissue at the bone-implant interface, and the foreign body reaction to wear particles. In vivo and in vitro studies employing biochemical, histochemical and molecular biological techniques have demonstrated that substances secreted from macrophages and possibly lymphocytes appear to modulate the functions of cells within the loosening membrane and the remodeling of the periprosthetic bone.

Purpose of the study

We report here our investigations of the effects of micromotion and particulate orthopedic materials on tissue differentiation within bone.

The specific aims were to assess:

1. the effects of short daily periods of micromotion on tissue differentiation.
2. the effects of several parameters of micromotion on tissue differentiation. These parameters include the configuration of the interface, the amplitude of micromotion and the number of daily motion periods.
3. whether terminating a motion protocol leads to alterations in tissue differentiation.
4. the effects of particulate orthopedic materials on tissue differentiation within bone.
5. the effects of micromotion versus implanted particulate high density polyethylene (HDPE) on tissue differentiation in bone.
6. whether T-lymphocytes are necessary for the initial recruitment of macrophages and foreign body giant cells to sites in which phagocytosable particles of HDPE have been implanted.

Experiments

MICROMOTION

These experiments were performed to assess the effects of short daily periods of externally applied micromotion on tissue differentiation within bone. Several parameters of motion, including the pore configuration, the amplitude and the number of daily motion periods were investigated. The effects of terminating a motion protocol known to produce primarily fibrous tissue were also explored.

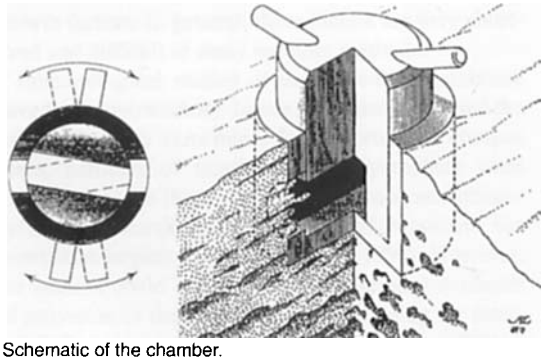
General methods

Description of the micromotion chamber

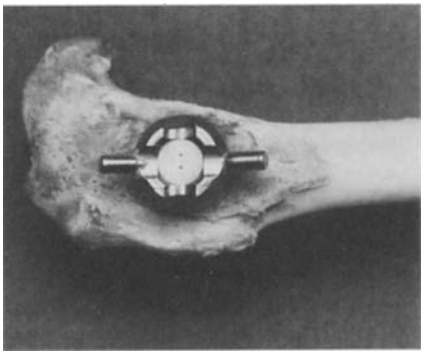
The micromotion chamber (MC) is a modification of the Bone Harvest Chamber (Albrektsson et al. 1984), which is described in the next chapter. The chamber was designed for implantation in the proximal, medial metaphysis of the rabbit tibia (Figure 1).

The MC is made of commercially pure titanium, and is composed of a fixed outer cylinder (which osseointe-

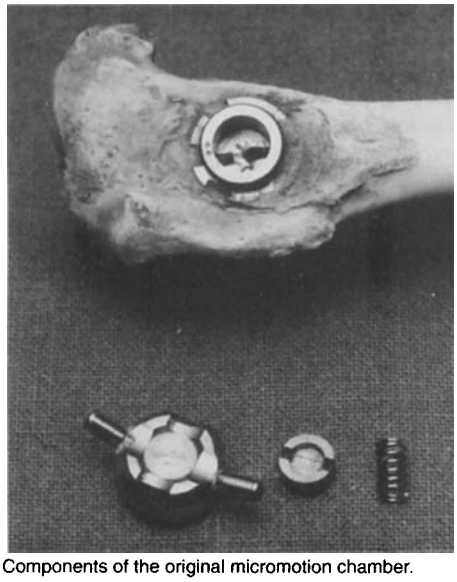
Figure 1. The micromotion chamber (see text for full description).



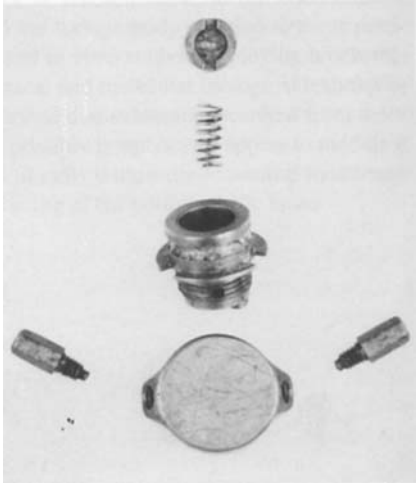
Schematic of the chamber.



The chamber implanted in the rabbit tibia.



Components of the original micromotion chamber.



The newer model of the micromotion chamber, used in later experiments.

grates with the surrounding bone), and an inner removable core. The outer cylinder is closed at one end (which is implanted into bone) and open at the other end (which faces the subcutaneous tissue) to receive the core. Both the cylinder and the core have a transverse 1 mm wide pore for tissue ingrowth. The inner core contains a 1 x 1 x 5 mm groove that coincides with the bottom plate of the outer cylinder and its holes, providing a continuous canal through the chamber. The outer cylinder undergoes osseointegration with the surrounding bone over a 6 week period. Thereafter, the bone within the chamber can be harvested at repeated intervals without disturbing the outer cylinder or surrounding bone.

The inner core of the MC is connected to a subcutaneous cover or cap that has two horns attached to it. The horns, which are palpable through the skin, are grasped by the researcher and manually rotated; this manipulation creates an ad latus motion in the canal at the interface between the core and the cylinder. A spring fits between the core and cover. The amplitude of movement is pre-determined by the size of a stopscrew.

Several different types of MC were used. The cylinders in the original MCs had 1 mm diameter round holes; later, cylinders with 1 mm square holes were fabricated (we did not anticipate the effect of pore configuration on bone ingrowth). The amplitude of motion was either 0.5 or 0.75 mm. In all experiments, the core was similar. Micromotions were applied at a rate of approximately 0.67–1 cycle per second.

Surgical procedure

Initial implantation of the micromotion chamber involves exposing the proximal, medial tibial metaphysis of the rabbit, just anterior to the medial collateral ligament. A hollow drill is used to excise a 6 mm round cortical window. The cylinder of the MC is screwed into bone with a special wrench so that the pores in the cylinder are at the level of the cortex of the tibia. The rest of the MC is then assembled. The tissues are closed in layers over the chamber.

After a 6 week period for osseointegration, the contents of the canal were harvested for the first time. From the third day after harvest, the chambers were manipulated on a daily basis. After 3 weeks, the chambers were harvested again. This regimen was followed repeatedly, frequently with intervening 3 week "rest periods" (in which no manipulations were performed) followed by a harvest.

Evaluation

The harvested tissue was fixed in formalin, decalcified, embedded in paraffin, and cut into 5-μm sections. In our first studies (I and III), multiple transverse sections were cut and stained with hematoxylin and eosin. Later studies (II, IV and V) employed multiple longitudinal sections enabling the entire length of the specimen to be visulaized on one slide.

The longitudinal and transvere sections were examined histomorphologically using 4× and 10× objectives. A qualitative assessment of the amount of bone in sections from the ends and the middle of the specimen was made in studies employing transverse sections (I and III). In later studies employing longitudinal sections (II, IV and V), 5 or more sections from each specimen were viewed for the histomorphological assessment.

Using an image analysis system, at first we quantitated the percentage of bone within 2 or more sections from each specimen using an image analysis system; with standardization of technique and practice, quantitations of different sections from the same specimen were witihin 5 percent of eachother. Thus, we subsequently performed the morphometric analysis on one longitudinal section from the middle of each specimen. Repeat morphometric analysis was carried out on approximately every tenth section, yielding results that were within 5% of the first assessment.

In some studies, four to eight hours prior to each harvest, the animals were given a known dose of 99-technetium methylene diphosphonate (^{99}Tc MDP), (approximately 45 MBq) intravenously. After surgery, the gamma emission of the harvested specimen was measured. The gamma emission of the specimen at time zero, the time of injection of the radiolabel, for a standardized dose of radiolabel was then calculated.

Statistics

Fisher's exact test, Wilcoxin's signed rank test (for paired data) and the Mann-Whitney test (for nonpaired data) were used. The Kruskall-Wallis test was applied when more than two groups were compared. Nonparametric statistics were used in the analysis because the number of animals in the studies was small and we could not assume that the percentage of bone ingrowth in this model had a normal distribution.

Experiments with a round-hole chamber

Methods

In I, the MC with an amplitude of 0.5 mm was implanted in six adult, lop-eared rabbits. This MC had a different "cap" or "cover" than was used in the subsequent studies. Furthermore, the "horns" were smaller. After osseointegration and harvesting, the chambers were assigned to either daily manipulations for 30 seconds, once daily, to produce 20 movement cycles, or no movement. After three weeks, the chambers were harvested. This regimen was followed repeatedly.

In II, MCs were implanted in five mature male New Zealand white rabbits. The amplitude of motion was 0.5 mm. After osseointegration and harvesting, the chambers were manipulated for 40 cycles per day ("40") and harvested after 3 weeks. The MCs were then manipulated at 40 cycles per day for three weeks and subsequently the manipulations were discontinued for three additional weeks ("40 + 0"); the contents of the chamber were harvested after 6 weeks. In the final time period ("0"), a harvest was performed after a 3 week period without any manipulation.

Results

In I, 7 of the 13 specimens harvested from moved chambers contained vascularized fibrous tissue, aligned longitudinally within the canal, without any visible cartilage or bone (Figure 2). 5 of the 13 specimens showed some ingrowth of woven or cancellous bone at one or both ends; only 1 of the specimens had scanty woven bone at the ends and in the middle. All of the 15 specimens harvested from control, nonmoved chambers contained some bone. 11 of the 15 specimens contained lamellar bone at the ends and in the middle of the specimen.

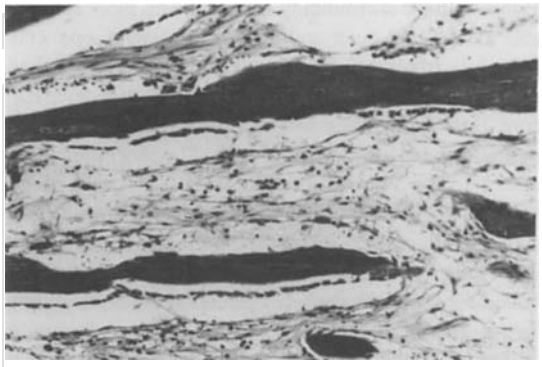
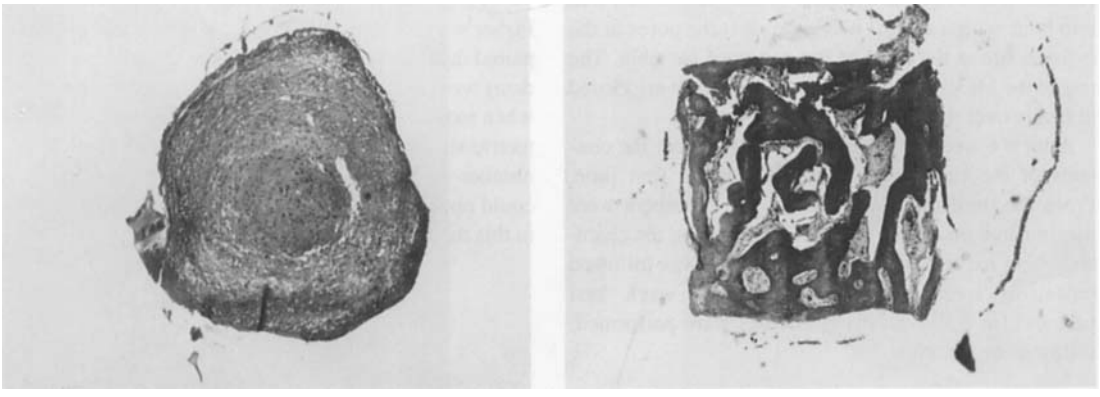


Figure 3. The tissue harvested from a non-manipulated chamber contained extensive bone in a fibrovascular stroma. Hematoxylin and eosin stain.

If each harvest is considered to be an independent event and the result is expressed as "no bone" versus "any bone" within the canal, the probability of a random occurrence of the result is 0.001 using Fisher's exact test (a similar result is found when the presence of bone in the middle of the canal is considered). If independence of the harvests is not assumed, the probability of a random occurrence of the result is still less than 0.05 using the Wilcoxin signed-rank test.

In II, histological sections from nonmoved chambers ("0") contained extensive trabecular bone,similar to previous non-moved controls (Figure 3). The "40"

Figure 2. Intermittent micromotion inhibits bone ingrowth using a round-hole chamber.



Transverse section of the contents of a chamber manipulated at 20 cycles per day. The specimen has a rounded shape. The fibrous tissue is dense in the center and more loose in the periphery. Hematoxylin and eosin stain.

Transverse section of the contents of a non-manipulated chamber. There is abundant trabecular bone present; the quadratic shape of the canal is preserved. Hematoxylin and eosin stain.

Figure 4. The harvested specimen from a chamber moved 40 cycles per day contained longitudinally oriented fibrous tissue with no evidence of inflammation. Hematoxylin and eosin stain.

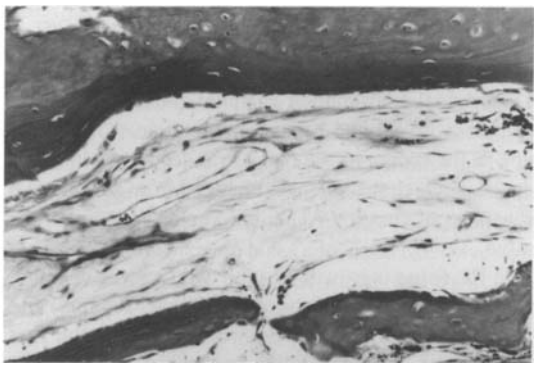


Figure 5. A specimen harvested from the group in which motion was discontinued, demonstrated findings similar to the non-manipulated group. Hematoxylin and eosin stain.

specimens were composed primarily of longitudinally oriented fibrous tissue and less bone compared to the "0" specimens (Figure 4). The trabecula appeared to be rather thin and short compared to the "0" specimens. The interstitium contained abundant fibroblasts, mesenchymal cells and capillaries. The "40 + 0" specimens were similar histologically to the "0" specimens (Figure 5).

The Kruskall-Wallis test was significant at $p < .01$. Bone ingrowth was less in the "40" specimens, compared to either the "0" or "40 + 0" specimens ($p = .01$) There was no difference between the "0" and the "40 + 0" specimens.

Conclusions

I. Using the round-hole chamber, one daily episode of motion (20 cycles per day) of a relatively short duration is adequate to inhibit bone ingrowth into the MC.

II. Discontinuing a motion protocol known to produce primarily fibrous tissue (rather than bone) within the chamber facilitated the ingrowth of new bone, and/or the differentiation of some of the existing fibrous tissue to bone.

Experiments with a square-hole chamber

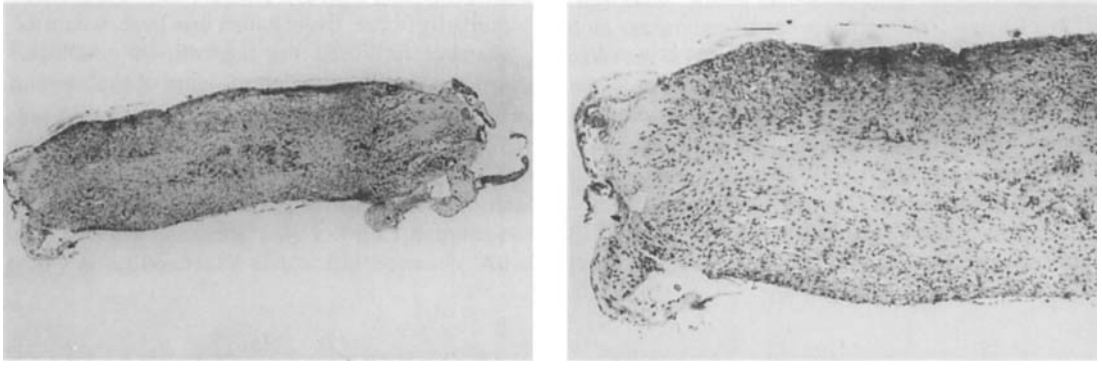
Methods

The original micromotion chamber contained an outer cylinder with a round 1 mm diameter hole. A modification in the design of the outer cylinder was then introduced in which the hole was changed from a round to a square configuration. The inner core remained the same.

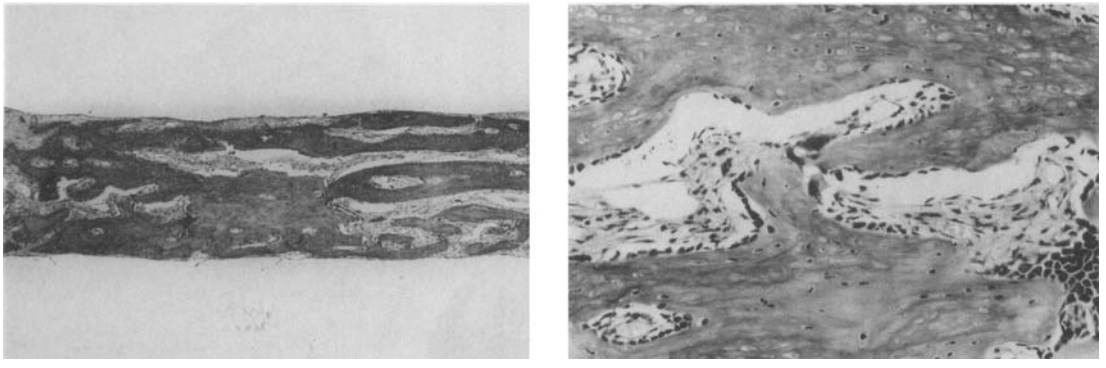
In III, tissue ingrowth into the two types of chamber was compared, using the same amplitude (0.5 mm) and frequency (20 cycles per day) of motion. In the first five rabbits, cylinders were implanted containing a round 1 mm outer hole. In the next four rabbits, the cylinders implanted were pierced by a 1 mm square hole; the inner core was the same. In the tenth rabbit, a cylinder containing a round hole was implanted in one leg; later a cylinder containing a square hole was implanted on the contralateral side.

In IV, the histological and scintimetric results of bone ingrowth into square-hole MCs having an amplitude of 0.5 mm versus 0.75 mm were compared. In both groups of animals, the frequency (20 cycles per day) and the configuration of the interface (square-holed cylinder and core) were the same. In the first five animals, micromotion chambers with an amplitude of 0.5 mm were implanted. Repeated harvests followed three week assignments to periods of motion or no motion. Two of the original 0.5-mm chambers were subsequently modified intra-operatively, during a harvesting procedure, without disturbing the outer cylinder or surrounding bone, to obtain an amplitude of 0.75 mm. This was accomplished by remachining the hole for the stop-screw that controlled the amplitude. In another 4 rabbits, a chamber with an amplitude of 0.75 mm was implanted primarily. The experimental protocol was continued with three week assignments to periods of motion or no motion.

Figure 6. Low power (left) and high power (right) photomicrographs of specimens from micromotion chambers undergoing 20 cycles/day. Hematoxylin and eosin stain.



Specimen from a *round-hole* micromotion chamber. The specimen contained vascularized fibrous tissue, aligned longitudinally in the canal, with little bone present



Specimen from a *square-hole* micromotion chamber. Note the extensive ingrowth of woven and trabecular bone in the section. Extensive bony remodeling is being carried out, as evidenced by the plump osteoblasts, and osteoclastic activity seen in the section.

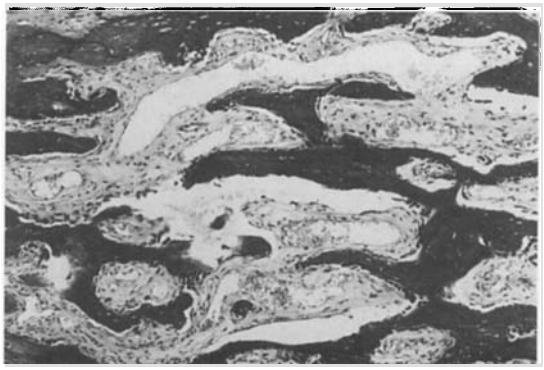


Figure 7. Photomicrograph of a specimen from a square-hole micromotion chamber undergoing 20 cycles/day with an amplitude of 0.75 mm. There is extensive ingrowth of woven and trabecular bone, which is being remodeled. The fibrous tissue stroma contains many capillaries. Hematoxylin and eosin stain.

In paper V, the histological and histomorphometric results of tissue ingrowth into MCs that were moved at 0 cycles per day, 20 cycles once per day, and 20 cycles twice per day for a 3 week period were compared. Seven MCs were manipulated daily using a frequency of 20 cycles per day. After three weeks, the chambers were harvested, then assigned to either another period of manipulation ("20") or no manipulation ("0"), and harvested again. This protocol was followed repeatedly. Later in the series, another manipulation protocol was included, namely 20 cycles delivered twice per day ("20 x 2"), once in the morning and once in the afternoon.

Results

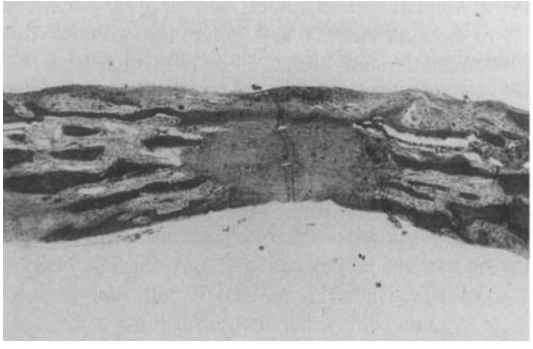
The histologic sections from the tissue harvested from round-holed chambers in paper III undergoing micromotion were similar to those treated similarly in papers I and II. The majority of these specimens contained vascularized fibrous tissue, aligned longitudinally in the canal, with little or no bone present (Figures 6). In contrast, all of the nine specimens harvested from cylinders containing square holes contained extensive new bone (Figure 6). The bone was woven and trabecular, and was surrounded by numerous osteoblasts.

A qualitative "bone ingrowth score" was calculated for each specimen based on the presence of bone in the outer one fifth of the specimen (grade 1), outer two fifths (grade 2), or throughout the specimen (grade 3). The average histological grade of the tissue harvested from cylinders with square holes was higher (more bone formation) compared to the tissue from cylinders with round holes ($p < .01$), whether independence of sequential harvests was assumed or not.

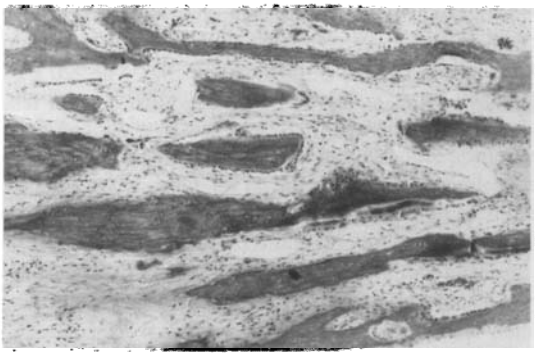
In paper IV, nine specimens were harvested in Group 1 (amplitude of 0.5 mm), and ten specimens were harvested in Group 2 (amplitude of 0.75 mm). Qualitative histology was similar in specimens from both groups, and contained extensive woven and trabecular bone, covered by osteoblasts (Figure 7). A trend was noted: chambers having a greater amplitude yielded less bone ingrowth compared to those with a smaller amplitude ($p < .08$).

Histological sections from nonmoved chambers in V contained extensive trabecular bone, generally aligned parallel to the long axis of the chamber (Figure 8). Unexpectedly, twenty movements per day appeared to further stimulate bone ingrowth. Extensive ingrowth of woven and trabecular bone was noted in a more cellular

Figure 8. Specimen from a nonmoved micromotion chamber. Note the extensive amount of trabecular and woven bone in a fibrovascular stroma. Hematoxylin and eosin stain.

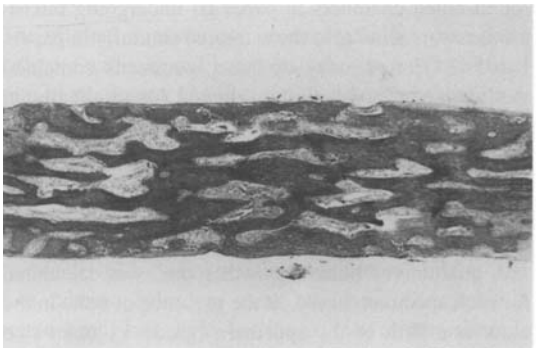


Low power.



High power.

Figure 9. Specimen from a square-hole micromotion chamber moved at 20 cycles once per day. Note that the bone ingrowth is more extensive; the trabeculae of bone are also thicker than in Figure 8. Hematoxylin and eosin stain.



Low power.

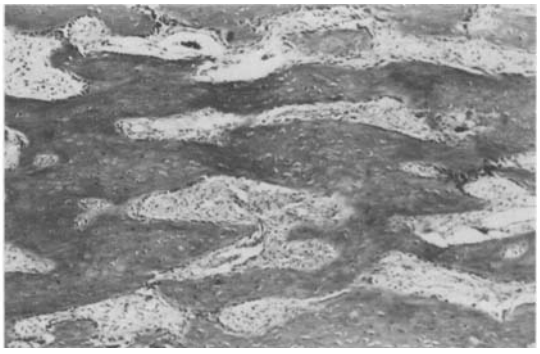


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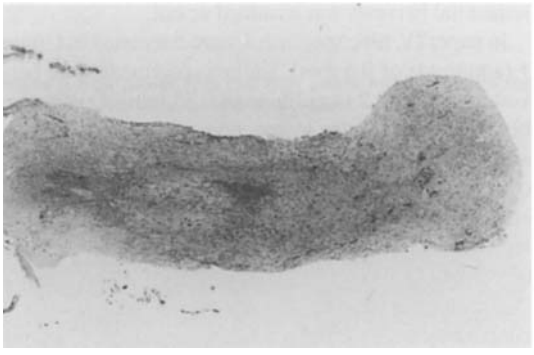
Figure 10. Specimen from a micromotion chamber moved 20 cycles twice per day. The section consists primarily of fibrous tissue. Hematoxylin and eosin stain.



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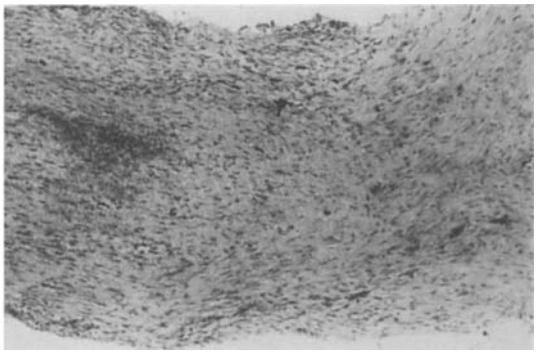


High power.

stroma (Figure 9). In general, increasing the degree of micromotion to 20 movements twice per day resulted in a decreased amount of bone formation. In several of these specimens, little or no bone could be found (Figure 10). Using the Kruskall-Wallis test, the morphometric data was significant at $p = .01$. There was more bone in the "20" group compared to the "0" group ($p = .03$) or the "20 x 2" group ($p = .02$). The "20 x 2" group specimens showed a tendency to contain less bone than the "0" group ($p = .06$).

Conclusions

III. A stimulus which depressed bone ingrowth in a round-holed chamber did not do so in a square-holed chamber.

IV. In a square-hole chamber, increasing the amplitude of motion from 0.50 to 0.75 mm favored tissue differentiation into fibrous tissue rather than bone.

V. In the square-hole chamber, a short daily period of low frequency (20 cycles per day) micromotion stimulated bone ingrowth; when the same micromotion was delivered twice daily, bone ingrowth was depressed.

Discussion

In the MC model, the ingrowing tissue is subjected to discrete, daily periods of manually imposed motion of a predetermined nature. The advantage of the model is that specific parameters of motion (e.g. amplitude, frequency, duration, pore size and pore configuration) can be applied in a controlled fashion. Furthermore, tissue specimens can be harvested repeatedly after the application of different motion parameters in the same site. Studies by our group and others, who employed fixed or moving titanium chambers implanted in the rabbit tibia have demonstrated no exhaustion effect for bone ingrowth after 20 or more harvests, if the animal is healthy (Albrektsson et al. 1984, Kälebo and Jacobson 1988) In our protocols, periodic, three week "rest" periods were intermittently used to monitor this effect. It is recognized, however, that the time in vivo of the chamber, and the fact that each animal did not receive an identical treatment regimen in some of the experiments, may have affected the results. Our MC model differs from the one described by Søballe et al (1992,1993), who employed a weight-bearing implant with numerous pores for bone ingrowth. In this model, the motion stimulus was dependent on the ambulation of the animal, and therefore the parameters of motion were not stringently controlled. Similar comments could be made concerning the two models described by Pilliar et al. (1986): in these studies, tissue ingrowth was observed in a weight-bearing, segmental femoral replacement and in a load-bearing tooth implant in dogs respectively. In another model (Hollis et al. 1992), rotatory motion was applied to multiple, porous coated titanium plugs implanted transcortically at different levels in the canine femoral shaft. The location of the plugs was, perhaps, less standardized and the duration of micromotion was much longer than in our study.

The MC is not a model that emulates the process of bone ingrowth into porous coated prostheses in man: the mechanics, surface morphology, and pore size of the MC are different. A limited number of acute strains are delivered to the tissue growing into the MC on a daily basis. The pore size of the MC is two to three times larger than those found in most conventional porous coated implants. The pore configuration is also different, as porous ingrowth devices have a more convoluted pore structure compared to the continuous, more uniform canal in our model. For these reasons, direct application of these experiments to the clinical situation should be cautioned. Despite this, the MC can provide insight into the effects of different parameters of motion on tissue differentiation within bone.

Using the MC with a round hole (Papers I and II), one daily episode of motion (20 or 40 cycles per day) of a relatively short duration inhibited bone ingrowth. This did not appear to be due to vascular disruption of the ingrowing tissue: gross bleeding was noted when moved specimens were incised at surgery. Furthermore, specimens viewed with a dissecting microscope demonstrated intact blood vessels traversing the specimens. It would appear that the strain generated by the movements of the MC either inhibited preosteoblast proliferation or influenced the pathway for a proliferated pluripotent cell population, favoring fibrous tissue rather than bone. It has previously been demonstrated that periods of micromotion can lead to the formation of fibrous tissue rather than bone, with regard to the healing of experimental osteotomies (Kenwright and Goodship 1990) and the ingrowth of bone into porous coated implants (Pilliar et al. 1986, Hollis et al. 1992). In I and II, it was shown that a micromotion stimulus of only 20 or 40 cycles per day delivered over a period of less than 1 minute was sufficent to inhibit bone ingrowth.

Recent studies have emphasized that mechanical stimuli such as the hydrostatic stress and distortional strain history (which are related to changes in the cellular pressure and shape respectively), appear to regulate the differentiation of mesenchymal tissues (Carter and Giori 1991, Giori et al. 1993). Stretching and distortion of connective tissue cells enhance the production of fibrous matrix, whereas hydrostatic pressure enhances the production of cartilaginous matrix by fibroblasts (Carter and Giori 1991). Randomly arranged fibroblasts and collagen fibers, when stretched longitudinally, become aligned along the direction of principal tensile strain (Harris et al. 1981, Nakatsuji and Johnson 1984, Klebe et al. 1989). Although it is beyond the scope of this study, it is noteworhty that the fibrous tissue formed after motion of the MC is regularly aligned, parallel with the canal, indicating longitudinal strains in the central part of the canal. The strain necessary to accomplish this effect may be small, because in non-moveable chambers (BHCs) which are harvested before bone ingrowth is complete, the same longitudinal fibrous tissue arrangement is seen, possibly due to strains caused by differences in elastic modulus between titanium and the surrounding tibial bone.

In paper II, when strains known to produce primarily fibrous tissue within the chamber were discontinued for three weeks, the new parameters of strain allowed the differentiation of the existing fibrous tissue to bone. Søballe et al. (1992, 1993) using a different micromotion model observed the conversion of a fibrous tissue interface surrounding a porous coated implant to bone 12 weeks after discontinuing the micromotion. Our

results suggest that a period shorter than 12 weeks may be equally effective in allowing the conversion of fibrous tissue to bone. Skerry et al. (1988), applying longitudinal strain to the isolated ulna in turkeys, showed that a short period of dynamic loading could alter the orientation of proteoglycan molecules in bone. This phenomenon was observed immediately after the application of the load and reversion to random orientation was complete within 48 hours after cessation of the load. Thus, mechanical forces appear to modulate the activites of mesenchymal tissue, in part, by their effects on the extracellular matrix. This mechanism may be more important for bone, compared to soft tissue, because the rigidity of the hard tissue matrix may not allow enough deformation for the cells to detect directly.

Despite being subjected to the same micromotion protocol, bone ingrowth was greater in the specimens from cylinders having square, rather than round holes (Paper III). It is possible that the different interface configurations may have influenced the type of tissue ingrowth. Since the moveable part of the bone ingrowth canal was always square, a square shape of the fixed part of the canal (square-hole chamber) creates a more congruent interface which might provide a more favorable milieu for bone ingrowth. Furthermore, the square hole in the cylinder also provided a larger cross-sectional area and thus a greater area for ingrowth of pluripotential cells at the interface. Other differences in the square and round hole experiments should be mentioned. The experiments using a round-hole chamber were performed first (when we were less experienced with the technique of manipulation), and the horns on these chambers were smaller and more difficult to grasp than with the square-hole chamber. Thus, the application of micromotion in the round-hole series may have taken a longer time than with the square-hole series. This may have produced a greater mechanical stimulus, resulting in less bone.

In Paper IV, we attemped to "overcome" the effects of interface congruity and increased cross-sectional area of the square-hole MC by providing a greater mechanical stimulus (increased amplitude) at the interface. Increasing the amplitude of motion from 0.50 to 0.75 mm appeared to favor tissue differentiation into fibrous tissue rather than bone.

In Paper V, the mechanical stimulus was increased even more by adding a second micromotion period per day, and, as expected, bone ingrowth was depressed.

Table 1. Summary of results of bone ingrowth into the micromotion chamber with different parameters of motion

Pore	Amplitude (mm)	Cycles per motion period	Motion periods per day	Bone ingrowth
Round	0	0	0	++
Round	0.5	20	1	+
Round	0.5	40	1	+
Round	0.5	40+0	1	++
Square	0	0	0	++
Square	0.5	20	1	++++
Square	0.75	20	1	+++
Square	0.75	20	2	+

The value for bone ingrowth represents a qualitative assessment of the percentage of bone within a section.

This study also demonstrated that a certain degree of micromotion can lead to an increase in bone ingrowth. On average, more bone was formed in specimens from square-hole chambers undergoing 20 cycles once per day compared to non-moved chambers or those undergoing 20 cycles twice per day. Although it has not been quantified, we also had an impression that the 20 cycles once per day treatment produced a different type of bone: instead of the fine network of trabeculae seen after "rest" periods, there were large areas of unremodeled, woven bone with a longitudinal orientation. Stimulation of bone formation by small movements has been reported by others, both in regards to fracture healing (Sarmiento et al. 1977, Wolf et al. 1981, Goodship and Kenwright 1985, Rubin and Lanyon 1985, Kenwright and Goodship 1990, Kenwright et al. 1991), and bone ingrowth into porous coated implants (Rubin and McCleod 1992).

Our studies (in addition to the ones referred to above) suggest the existence of a window of externally applied mechanical strain, that may modulate the differentiation of mesenchymal tissue into fibrous tissue or bone. Low levels of strain may enhance bone formation; however when a critical strain level is exceeded, bone formation is depressed. Our studies also confirm that the mechanical stimulus need only be applied for a short period each day to affect tissue differentiation. Furthermore, from II, it would appear that the strain history is constantly updated, enabling the adaptation to changes in the current functional biomechanical environment.

Table 1 summarizes the major findings from our micromotion studies.

PARTICULATE MATERIALS

The purpose of these experiments was to assess the effects of particulate orthopedic materials (bone cement, high density polyethylene, titanium 6-aluminum 4-vanadium alloy and cobalt-chrome alloy) on tissue differentiation within bone.

Description

The Bone Harvest Chamber (BHC) is the "grandfather" device of the MC, is also made of commercially pure titanium (Figure 11), and is implanted in the proximal, medial tibial metaphysis of rabbits (Albrektsson et al. 1984, Kälebo and Jacobson 1988). The three main components of the chamber are the outer cylinder, the inner core, and two threaded screws that connect the cylinder and core. The cylinder is pierced by two transverse, round, 1 mm diameter holes or pores. The core in the MC and BHC are similar, except there is no provision for movement in the latter. When the chamber is assembled in bone, there exists a continuous canal through the implant for tissue ingrowth.

Surgical procedure

The initial implantation of the BHC was similar to that described for the MC, except that the chambers were implanted bilaterally. Six weeks later, the contents of the canal were harvested for the first time. Particulate materials, in a carrier, were inserted into the chambers unilaterally or bilaterally. After 3 weeks, the chambers were harvested again. After each harvest, the cores and the inner portion of the cylinders were thoroughly cleansed with physiologic saline to remove any remaining material. This regimen was followed repeatedly, frequently with intervening 3 week "rest periods" followed by a harvest.

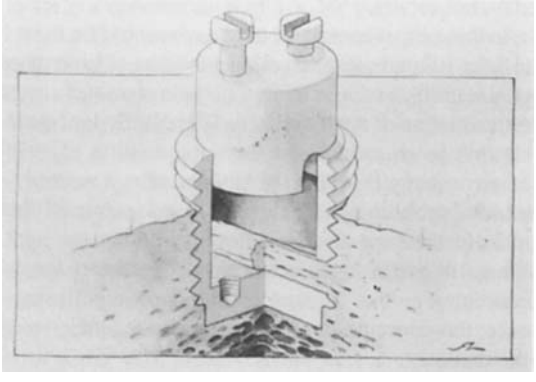


Figure 11. The bone harvest chamber.

Particulate materials

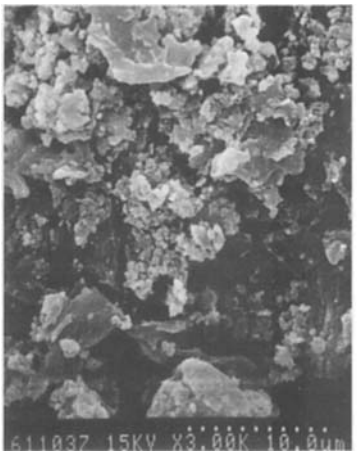
We attempted to use particles similar to those observed in retrieved membranes. The majority of the particles were less than 10 μm in diameter and could therefore be phagocytosed by histiocytes and giant cells. The size of the particles was measured on a scanning electron microscope interfaced with a computer morphometric image analysis system, and/or by laser scatter analysis (Spectrex Corp., Redwood City, CA). The particulate materials for these studies included the following (Figure 12):

Bone cement particles. The fabricated particles of bone cement (BC) were made at the Harrington Arthritis Research Center in Phoenix, AZ., USA from Simplex Surgical Cement (Howmedica, Rutherford, NJ, U.S.A). The bulk cement was cured, crushed and sifted to obtain particles approximately 10 μm in size. Using laser scatter analysis, the particles averaged 3.54 μm in diameter (standard deviation = 6.59) (Emmanual and Hedley 1991).

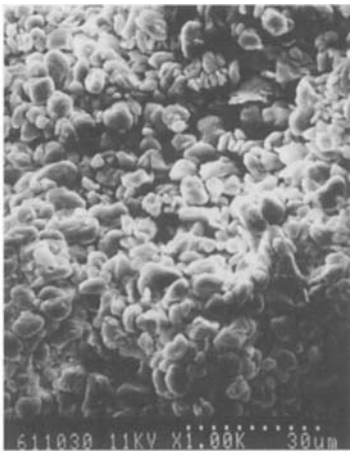
Polyethylene particles. The generation of particles of ultra high molecular weight polyethylene (UHMWPE), the material currently used for total joint replacement has been a very difficult task; recently this has been performed using an attrition technique, yielding fractions of different densities and with crystalline and amorphous phases (Leigh et al 1992). These UHMWPE particles have not yet been fully characterized, and are not for general distribution to orthopedic research laboratories. We could obtain (and subsequently used) small phagocytosable particles of high density polyethylene (HDPE); this material is similar to UHMWPE, but is slightly more dense and has a lower molecular weight. The HDPE particles were a gift from Smith and Nephew Richards, (Memphis TN, U.S.A). These particles were produced by Shamrock Technologies, (Newark, NJ, USA), and were reported to be 100% pure, highly crystalline with specific gravity 0.95. The particles were globular shaped and averaged 4.7 ± 2.1 μm (mean ± standard deviation) using scanning electron microscopy.

Titanium alloy particles. The particles of titanium alloy (Ti) were produced by cyclic loading and resultant fretting of titanium 6-aluminum 4-vanadium alloy modular hip components in saline in the laboratory . The fretting conditions were intentionally chosen to produce sufficient quantities of metallic particles for the study. The particles were a gift from Smith and Nephew Richards, (Memphis, TN, U.S.A). The size of the particles was measured on a scanning electron microscope interfaced with a computer morphometric image analysis system and averaged 3.0 ± 2.6 μm.

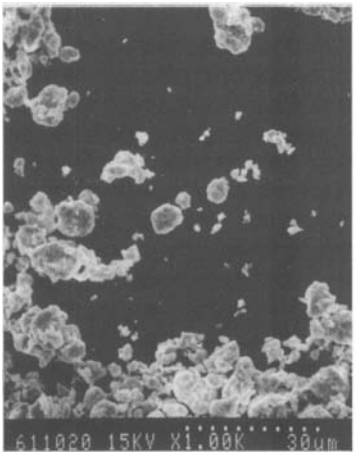
Figure 12. Scanning electron microscopic photographs of some of the particles used in this study. (Photographs were produced by Richard L. Landingham and Jim Yoshiyama, Lawrence Livermore Laboratory, Livermore, California U.S.A.)



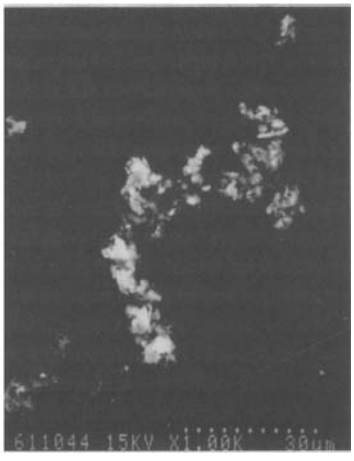
Bone cement particles. The dotted line at the bottom of this figure represents 10 µm.



High density polyethylene particles. The dotted line represents 30 µm.



Titanium 6-aluminum 4-vanadium alloy particles. The dotted line represents 30 µm.



Cobalt chrome alloy particles. The dotted line represents 30 µm.

Cobalt-chrome particles. These particles were a gift from Zimmer Inc. (Warsaw, IN, U.S.A.), and were produced by milling and sieving of an alloy composed of cobalt-chrome-molybdenum (CoCr) used for making hip prostheses. These particles averaged 2.7 ± 2.1 µm .

All particles were gas sterilized with ethylene oxide and allowed to aerate for at least 24 hours prior to implantation. The carrier solution for the particles was 1 percent sodium hyaluaronate (Healon). Healon was mixed with the particles, using a sterile spatula, immediately prior to implantation. The Healon/biomaterial composite was introduced via a needle and syringe to fill the groove in the core of the BHC.

In these experiments, we used a concentration from 1 to 2.5 x 10^8 particles / mL . Using the data of Livermore et al. (1990), and assuming a particle size of 5 µm, a concentration of approximately 10^8 polyethylene particles/mL is an estimate of the concentration of polyethylene particles within the hip joint after a wear period of approximately 1 year, if it is also assumed that most of the generated particles stay within the joint. Thus, 10^8 particles/mL is a reasonable estimate for the concentration that the surrounding bone might experience; this concentration was chosen for the majority of the studies.

Evaluation

The harvested tissue was fixed in formalin, decalcified, embedded in paraffin, cut into 5 µm longitudinal sections and stained with hematoxylin and eosin. The histomorphological and morphometric studies were similar to those described for the MC.

Statistics

Statistical analysis was performed using the Wilcoxin signed rank test (for paired data) and the Mann-Whitney test (for non-paired data). The Kruskall-Wallis test was applied when more than two groups were compared.

Experiments

Methods

In paper VI, two experimental protocols were employed and two concentrations of BC particles were used. In the first series of six rabbits, Healon was mixed with BC particles using a concentration of 2.5 x 10^8 particles /mL. The contralateral chamber was left empty and served as a control. After harvest 3 weeks later, this "particles versus control" implantation was repeated with a switch of sides, and final harvest was done after another 3 weeks. Experiment and control sides were compared. In the second series of six rabbits, implantation was carried out sequentially using the same material (Healon with/without BC) bilaterally. The concentration used was 1 x 10^8 particles/ml. The tissue within the chamber was harvested at three weekly intervals and experimental harvests were compared with control harvests.

In paper VII, Healon was implanted bilaterally in the chambers, after the first harvest. In subsequent implantations, Healon was mixed with particles of HDPE or Ti to form a concentration of 1 x 10^8 particles /mL. The test materials was implanted unilaterally. The contralateral chamber was left empty and served as a control. The chambers were harvested repeatedly, alternating experimental and control sides. The values for the percentage of bone ingrowth were standardized for each chamber by calculating the "bone ingrowth ratio," which divides each value for the percentage of bone ingrowth by the corresponding entry when Healon alone was implanted in the same chamber. After this standardization, the experimental and control sides were compared.

In paper VIII, eleven rabbits were divided into two series of animals in order to test whether the order of implantation had an effect on the outcome. The first part of the protocol was common to both series. After the first harvest, Healon was placed in the cores bilaterally; a harvest was performed three weeks later. Thereafter, in the first series of five animals, CoCr particles, dispersed in Healon carrier, were inserted into the core of the chamber. After three weeks, the chambers were harvested again. This was followed by a three week "rest" period without any material implanted in the chamber. After another harvest, HDPE particles, dispersed in Healon were implanted; the tissue in the chamber was harvested three weeks later. The second series of six animals followed an identical protocol except that the order of the materials was reversed: HDPE particles were implanted after Healon, and CoCr particles were implanted after the "rest" period. Again, only harvests from the same chambers were compared.

Results

In VI, the sections from the control harvests, and those containing Healon alone contained extensive trabecular bone arranged longitudinally in the canal, in a fibrovascular stroma (Figure 13). The sections containing BC particles were infiltrated by foamy, mononuclear and multinuclear histiocytic cells, surrounding and engulfing the larger cement "ghosts" and smaller particles (probably barium sulfate which is not dissolved during routine processing) (Figure 14). Healon plus bone cement particles was associated with less ingrowth of bone compared to the non-implanted controls or Healon alone ($p = .01$).

In VI, the sections containing titanium alloy particles were qualitatively and quantitatively similar to the control sections and those containing Healon, except for the presence of small black granules of titanium alloy, dispersed in the fibrovascular stroma or phagocytosed by scattered macrophages (Figure 15). The sections containing HDPE particles were infiltrated by mononuclear and multinuclear histiocytic cells in a highly fibrous stroma (Figure 16).

Using the standardized "bone ingrowth ratio" for each entry, HDPE particles were associated with a decrease in bone ingrowth compared to the contralateral controls ($p = .03$), and Ti particles ($p = .03$). Ti particles did not decrease the bone ingrowth ratio when compared to their respective controls ($p = .60$). No differences were noted in the control harvests opposite the Ti and HDPE particles respectively ($p = .75$).

A similar histological response to implanted HDPE was observed in papers VII and VIII (Figure 16). When CoCr alloy particles were implanted, the tissue exhibited a more florid foreign body and chronic inflammato-

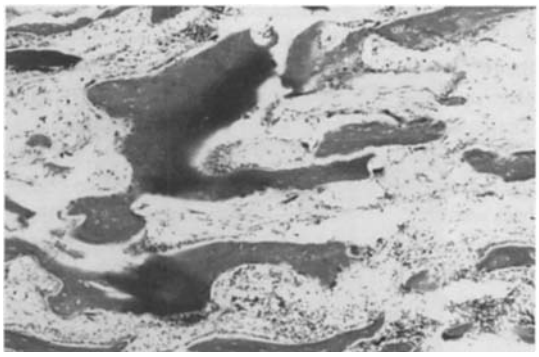


Figure 13. Sodium hyaluronate (Healon) specimen. These photomicrographs demonstrate extensive ingrowth of trabecular and woven bone in a fibrovascular stroma. Hematoxylin and eosin stain.

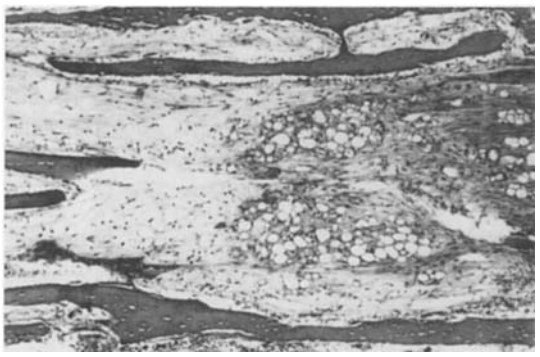
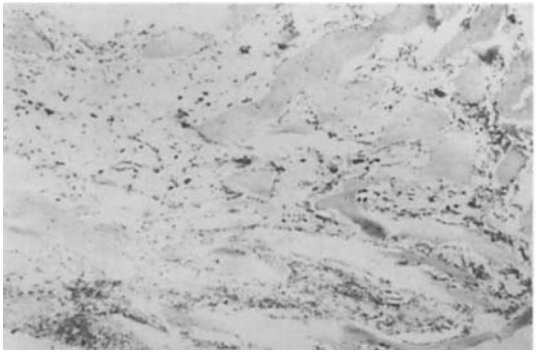


Figure 14. Specimen containing sodium hyaluronate (Healon) and cement particles. Large areas in this section are infiltrated by foamy mononuclear and multinuclear histiocytic cells associate with round polymethylmethacrylate "ghosts". Bone ingrowth is less extensive. Hematoxylin and eosin stain.

Figure 15. Section containing titanium alloy particles. The black granules of titanium alloy are dispersed throughout the section; some of the particles are located within scattered macrophages but there is little evidence of foreign body or chronic inflammatory reaction. The trabecular bone appears normal. Hematoxylin and eosin stain.



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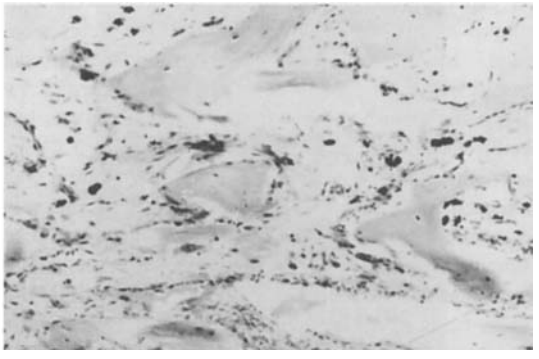
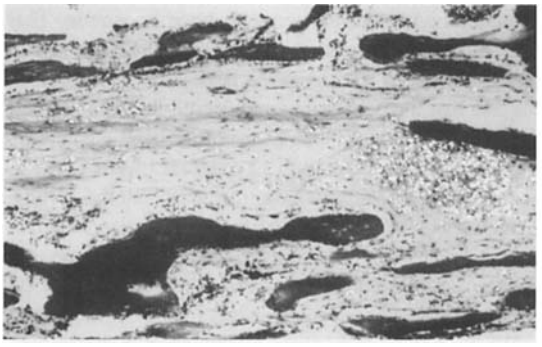


High power

ry response, often in a nodular arrangement, in a background of dense connective tissue (Figure 17). Bone ingrowth was sparse and areas of hyaline degeneration were noted in the CoCr specimens. Intracellular particles were observed in both the HDPE and CoCr specimens.

To account for chamber to chamber differences, the values for bone ingrowth for each treatment were divided by the corresponding value when Healon alone was implanted. Using this method of standardization, no relationship was found between the percentage of bone ingrowth and the order in which the different particles were implanted. Therefore, we combined the results of series 1 and 2, and performed a statistical analysis for the four treatments (Healon, cobalt chrome alloy particles, rest, HDPE particles) using 11 animals. The Kruskall-Wallis test was significant ($p = .0001$). Intergroup comparisons using the Wilcoxin signed-rank test demonstrated that the sections containing particles of cobalt chrome alloy were associated with less ingrowth of bone compared to those implanted with Healon ($p = .005$), nothing ("unimplanted") ($p = .003$), or polyethylene particles ($p = .01$). The addition of polyethylene particles also decreased the amount of bone ingrowth into the chamber, when compared to the Healon ($p = .005$) or "unimplanted" ($p = .01$) treatments. There was no difference between the values for the Healon and "rest" treatments ($p = .65$).

Figure 16. Section containing HDPE particles. The positively birefringent particles of HDPE are surrounded by histiocytes and chronic inflammatory cells in a fibrous stroma. Trabecular bone is less prominent compared to the control section. Hematoxylin and eosin stain.



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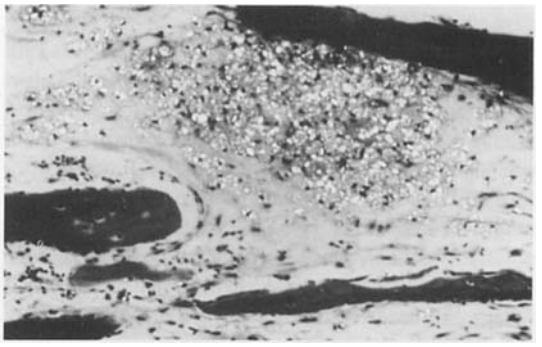

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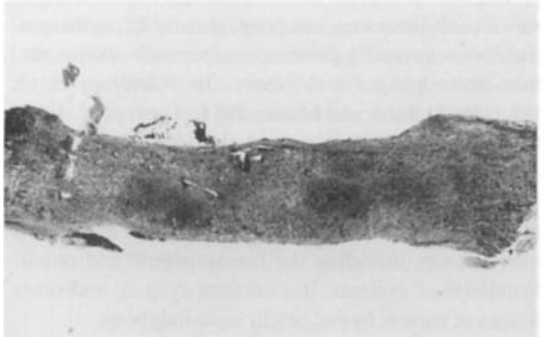
Figure 17. A specimen harvested from a chamber containing cobalt chrome alloy particles. Note the florid foreign body and chronic inflammatory reaction, often arranged in nodules. Hematoxylin and eosin stain.



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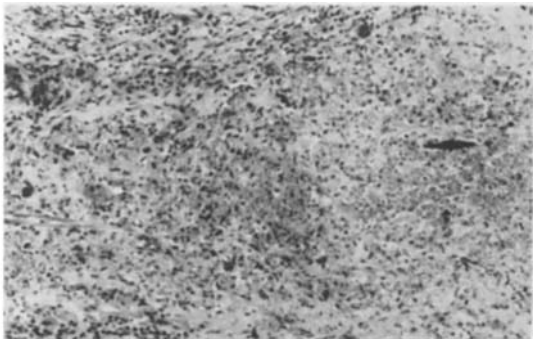

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Conclusions

VI. Concentrations of 1–2.5 x 10^8 particles /mL of bone cement are associated with a foreign body response and diminished net formation of bone within the Bone Harvest Chamber.

VII. Polyethylene particles appear to be more deleterious to net formation of bone in the Bone Harvest Chamber, compared to particles of titanium alloy.

VIII. Cobalt chrome particles produced a more florid foreign body and chronic inflammatory reaction, and were associated with decreased ingrowth of bone compared to particles of polyethylene.

Discussion

Wear debris is produced, to some degree, from every joint prosthesis, due to the properties of the materials used, and the presence of motion at the articulation and at the different interfaces. Particles of bone cement may be implanted initially during surgery, due to inadequate mixing of the monomer and polymer constituents of the cement (Goodman 1989b). Cement debris may also form during cyclic loading of the implant, due to fatigue fracture or abrasive wear of the cement with the prosthesis or bone (Jasty et al. 1991). Metal particles and their byproducts are generated from the metal-plastic articulation, from fretting of the metallic stem with cement or bone, and from the interfaces between different (modular) parts which comprise one articulating component (Collier et al. 1991, 1992, Mathiesen et al. 1991, Betts et al. 1992, Kovacs et al. 1992). Particles of polyethylene are generated directly from the metal-plastic articulation, from the interface between polyethylene and metal backing or adjacent screws, and via third body wear (e.g. due to cement particles entering the articulation) (Nusbaum et al. 1979, Rose et al. 1979, Bartel et al. 1986, Goodman and Lidgren 1992).

The foreign body reaction associated with bone cement and polyethylene particles in the Bone Harvest Chamber was similar to the findings in other animal models, and in retrieved membranes from failed, polyethylene-bearing joint replacements in humans (Bullough 1973, Vernon-Roberts and Freeman 1976, Willert and Semlisch 1977, Mirra 1982, Linder et al. 1983, Goodman et al 1988b, Howie et al. 1988, Goodman et al. 1990, Goodman et al. 1991b, Spector et al. 1990). Howie and Vernon-Roberts (1988) have reported similar findings to ours when cobalt chrome particles were implanted in the rat knee joint. *In vitro* studies have also pointed to the potential toxicity of CoCr particles in cell culture (Mital and Cohen 1968, Pappas and Cohen 1968, Rae 1975, 1981). This reaction was not observed when slightly larger CoCr particles were implanted in the medullary canal of the rabbit tibia (Goodman et al. 1990b). Clearly, the location and the characteristics of the implanted particles are important determinants of the histological reaction. Some of the important properties of the particles include the chemical composition, size, shape, topography, the surface energy and surface charge (Nagura et al. 1977, Besterman and Low 1983, Kawaguchi et al. 1986, Tabata and Ikada 1988, Perry et al. 1990). It was not the purpose of our study to examine all of these properties, but to assess the effects of similar-sized particles of materials used for orthopedic implants.

The particles of bone cement, polyethylene and CoCr alloy, at the concentration used in this experiment were associated with decreased net formation of trabecular bone. Interestingly particles of titanium alloy, were not associated with a decrease in net bone formation. The decrease in bone ingrowth is not simply a "space occupying effect" of the material itself; our calculations suggest that the particles would occupy less than 5 percent of the volume of the canal of the BHC. It would appear that bone ingrowth is mitigated by the space occupied by the cellular response associated with the particles, by the liberation of factors that decrease the formation and/or enhance the degradation of bone, or, in the case of bone cement, due to the toxic effect of residual monomer.

What is the mechanism by which polymeric and metallic debris modulate bone formation? It has been shown that macrophages become activated when they phagocytose particles (Williams et al. 1984, Van Oss 1986, Johnson 1988, Murray and Rushton 1990). This produces a cascade of events leading to the release of various substances such as prostaglandin E2, cytokines, leukotrienes, metalloproteases, superoxide anions etc. from macrophages and other cells (Goldring et al. 1983, 1988, Raisz and Martin 1984, Appel et al. 1988, Kim et al. 1988, Goodman et al. 1989, Mather et al. 1989, Ohlin et al. 1990, Kossovsky et al. 1991, Chiba et al. 1992). These substances regulate the growth, proliferation, differentiation and degradation of the cells of many tissues including the hematopoietic and reticuloendothelial systems, the immune system, and other tissues of mesenchymal origin including bone.

The changes in net bone formation noted with the various particulate materials could have been due to increased degradation of bone, decreased formation of bone, or both. Glowacki et al (1986) have previously shown that polyethylene particles implanted in subcutaneous pockets in rats elicited increased numbers of foreign body giant cells. In contrast, the multinucleated cells that were associated with the implantation of bone particles of a similar size to the polyethylene stained positively for tartrate resistant acid phosphatase (TRAP) and were also interpreted as being osteoclasts morphologically, using electron microscopy. In the current studies, bone cement, polyethylene and CoCr particles have been associated with a decrease in net formation of bone. From the work of Glowacki et al, we assume that the multinucleated cells elicited by these particulate materials are foreign body giant cells rather than osteoclasts. If this assumption is correct, the histological appearance gives no evidence that bone resorption is increased. On the contrary, we believe that an inhibition of osteoblast activity has occurred, because bone was absent in areas where polyethylene particles

had become more concentrated. In this model, it is unlikely that bone had first been formed and then totally removed in these areas at three weeks; rather, formation never took place. We are currently attempting to refine our explanation concerning the mechanism of decreased net formation of bone in the presence of particulate materials by utilizing histochemical and immunohistochemical staining techniques.

The particle sizes and concentrations used in this study attempted, in a simplified way, to parallel the circumstances seen clinically and histologically in aseptic loosening of joint arthroplasties. With respect to the size of the particles, these were usually less than 10 μm and capable of being phagocytosed; however, recent observations have stressed the importance of even smaller particles that are beyond the resolution of the light microscope. These particles may be in much greater numbers and be more easily phagocytosed than particles as large as 10 μm (Campbell et al. 1992). The shape of the particles used in this study may also be different from that of the particles generated in vivo, in humans. We also recognize the fact that we used high density polyethylene, rather than ultra high molecular weight polyethylene (the material currently used for joint arthroplasty) for our experiments. Thus, direct application of our findings to the clinical situation is speculative.

From this study, we may hypothesize that the presence of excessive amounts of metal and polymer debris may inhibit the ingrowth of bone into porous coated joint arthroplasties. Although it is unlikely that a sufficient amount of such debris would normally be generated immediately after a porous coated prosthesis is implanted, this scenario could possibly be important in situations in which the implant was defective or damaged during prosthetic insertion, or in cases of third body wear. Furthermore, it is not uncommon to implant a porous coated prosthesis when revising a failed cemented or noncemented component. Possibly, remaining metal and polymer debris may be important in decreasing bone ingrowth in the revision situation, especially if the "pseudomembrane" is not thoroughly debrided. Once the prosthesis is in place, the ongoing generation of new debris may also have implications for the remodeling process of the prosthetic bed, promoting both increased degradation and decreased formation of bone.

Tissue differentiation in the presence of micromotion or particulate polymer materials

In this experiment we contrasted the effects of micromotion, versus phagocytosable particles of two orthopedic polymers on tissue ingrowth into the MC.

Methods

In paper IX, the round-hole MC was implanted into the proximal right tibia of 5 mature male New Zealand white rabbits. After the first harvest, the chambers were manipulated at 40 cycles per day, using an amplitude of 0.5 mm. The tissue within the chamber was harvested after 3 weeks. In the following series, fabricated particles of BC or HDPE were mixed with Healon to obtain a concentration of 1 x 10^8 particles/ mL; this solution was implanted in the canal of the chamber but micromotion was not instituted. Harvesting of the contents of the chamber was performed at three weekly intervals. The histomorphological and morphometric studies were similar to those described for the BHC. Statistical analysis was performed using the Kruskall-Wallis test.

Results

Histological findings from specimens harvested from nonmoved chambers, those implanted with Healon, BC or HDPE, and those undergoing 40 cycles per day ("40") of micromotion were similar to those described in II, VI and VII. The rankings for the mean percentage of bone in the section, for the different interventions were, in decreasing order: 0 cpd > Healon > BC > "40" > HDPE (Table 2). The Kruskall-Wallis test was significant at $p = .001$. Chambers containing cement or HDPE particles, or undergoing motion exhibited less trabecular bone compared to nonmoved chambers or those containing Healon alone ($p < .05$). The HDPE and "40" treatments resulted in less bone compared to the cement treatment ($p < .05$).

Table 2. Relative effect of particulate materials and micromotion on bone ingrowth into the titanium chambers

Stimulus	Bone ingrowth
Rest	++++
Healon	++++
Ttitanium alloy particles	++++
Cement particles	+++
Polyethylene particles	++
40 cycles/day	++
Cobalt chrome alloy particles	+

Note: particles are mixed with Healon at a concentration of 1 x 10^8 particles /mL

Conclusion

The previous effects of 40 cycles per day of micromotion and polymeric debris were confirmed. Micromotion and HDPE particles were equally effective in depressing net formation of bone, using this model.

Discussion

This study showed that, despite evoking different histological reactions, both micromotion and polymeric particles have an adverse effect on net bone formation in the rabbit chamber model. Mechanical (micromotion) and chemical (the release of soluble factors from stimulated macrophages) stimuli both appear to modulate differentiation or function of mesenchymal cells.

From the present studies, we are able to rank the relative ability of different materials and mechanical stimuli to affect net bone formation (Table 2).

The role of T lymphocytes in macrophage recruitment to polyethylene particles

The purpose was to determine whether T cells are necessary for the initial recruitment of macrophages to phagocytosable particles of high density polyethylene implanted in bone.

Methods

After aspirating 0.1 mL of bone marrow, a bolus of 3 x 10^7 particles of high density polyethylene mixed in 0.1 mL of the carrier sodium hyaluronate was injected into the right proximal tibia of 10 normal and 10 T cell deficient (nude), Rowett rats from the same litters. The particles of HDPE averaged 4.7 ± 2.1 μm. A similar procedure was performed on the left side of the animals, except sodium hyaluronate alone was injected. The procedures were performed percutaneously under sterile conditions. The animals were killed after six weeks. Transverse, paraffin embedded sections stained with hematoxylin and eosin were made of the implant area.

Results

3 of the normal rats died immediately post-operatively due to an anesthetic overdose. 2 of the T cell deficient rats died four weeks post-operatively; 2 of the normal rats were therefore killed at this time for comparison. The remaining animals survived without complications.

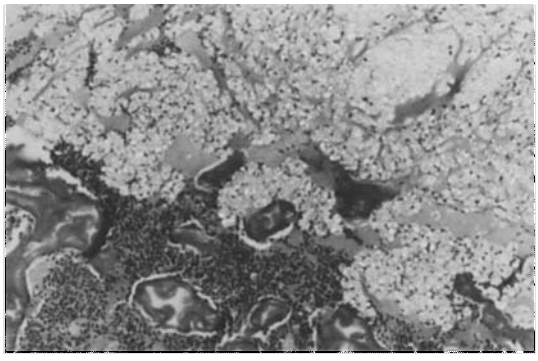
The right tibiae from the rats that died immediately postoperatively contained positively birefringent HDPE particles intermingled with normal marrow cells. The left side contained light pink staining localized areas, devoid of cells, consistent with sodium hyaluronate, intermingled with normal appearing marrow cells. No acute or chronic inflammatory or foreign body reactions were identified. At 4 and 6 weeks, the tibiae injected with sodium hyaluronate demonstrated the presence of normal bone marrow. In both normal and T cell deficient rats, macrophages were noted to surround and engulf the HDPE particles in the right tibia (Figure 18). No differences were noted in the histological reactions to HDPE particles implanted in the two groups.

Conclusion

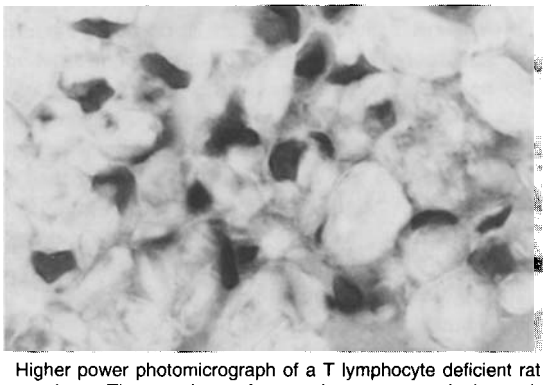
T lymphocytes are not necessary for the initial recruitment of macrophages to sites in which phagocytosable particles of HDPE have been implanted in the rat.

Figure 18. Implantation of HDPE particles in normal and T cell deficient rats.

Normal rat specimen. The medullary cavity contains marrow cells and bone. The positively birefringent polyethylene particles are surrounded and engulfed by macrophages. Hematoxylin and eosin stain.



T lymphocyte deficient rat specimen. Note that the macrophages are seen in a similar relation to the positively birefringent polyethylene particles as seen in the normal rats.Hematoxylin and eosin stain.



Higher power photomicrograph of a T lymphocyte deficient rat specimen. The cytoplasm of macrophages surrounds the positively birefringent polyethylene particles, which indent and the deform the nuclei. Hematoxylin and eosin stain.

Discussion

Since our study was performed, Jeranik et al. (1993) have provided evidence that T and B lymphocytes as well as NK cells (activated killer cells) are not necessary for macrophage accumulation to an area implanted with polymethylmethacrylate particles. Whether the macrophages function normally in the absence of T lymphocytes is unknown. It is well established that lymphokines play a critical role in modulating the release of inflammatory mediators from macrophages (Unanue and Allen 1987, Johnston 1988). In studies by Rentz et al. (1989), the release of the macrophage products TNF-a, IL-1 and PGE_2 from nude rats was less than that from euthymic rats in an experimental model of acute and chronic arthritis. Clinically, the inflammatory response was less severe, and acute but not chronic arthritis developed in the nude rats. If similar mechanisms pertain to the macrophage response to particulate debris, then the modulating influence of T lymphocytes would appear to be important.

General discussion

Loosening, micromotion and particulate debris

Revision of joint arthroplasties due to aseptic loosening still constitutes a major problem. This problem may be compounded by the fact that new, "advanced" prosthetic designs and materials are being introduced to the marketplace expeditiously, often before comprehensive experimental and clinical evaluations (in selected centers) have been completed (Bauer 1992, Goodfellow 1992). In this repect, the use of national registries to analyse the outcome of different prostheses and techniques should facilitate a more unbiased assessment of our surgical interventions (Lindstrand et al. 1992, Malchau et al. 1993). In fact, there is little evidence that any of the new prostheses are superior to the one introduced by Charnley a quarter of a century ago.

Loosening of joint prostheses may be due to a combination of interacting mechanical and biological factors (Jasty et al. 1991, Schmalzried et al. 1992, for review see Goodman and Lidgren 1992). Much information concerning the mechanics and biology of prosthetic failure has been obtained from clinical studies in humans, and from retrieval studies of the implants and tissues surrounding the prosthetic components. However, due to the complex nature of joint replacement and the multitude of patient variables, it is often difficult to identify specific factors that may have a direct effect on the outcome of an implant until a large number of cases have been analysed. *In vitro* studies can provide important insights into specific problems associated with prosthesis implantation, such as the biochemical effects of particles of a certain size and shape on a uniform population of cells. However, the nature of these experiments creates an artificial environment that is different from the human body in which these events supposedly occur; furthermore, these events in humans usually take place over many months or years, rather than hours to days in *in vitro* experiments. Engineering studies are important in the selection and evaluation of the materials and prosthetic designs, but can not simulate all the biological processes associated with prosthetic implantation in the body.

Histological and biochemical data have been reported from several *in vivo* models that simulate the processes associated with prosthetic loosening (Spector et al. 1990, Goodman et al. 1992). These models might be criticized on many grounds, e.g. particles are added immediately, and the prostheses are clinically loose at surgery. Furthermore, the adequacy of the controls in these studies is questionable. These models engender more basic questions concerning how to simulate prosthetic loosening in humans. Questions pertain to the selection of the appropriate experimental animal, location, and surgical procedure, the specific method of simulating loosening, the determination of what data to collect and how to interpret it, and the error associated with measurement.

It was our purpose to develop a model to explore the general principles that govern tissue differentiation into a single pore within bone in the presence of motion and particulate materials, rather than to model a single clinical situation, in which one can not avoid the influence of several biological and mechanical factors. The Bone Harvest Chamber and Micromotion Chamber afford an opportunity to study the effects of specific variables on tissue differentiation within bone, in a relatively uniform population of animals. The models are simple, inexpensive, reproducible, and have defined, quantifiable endpoints for data analysis, compared to other models (Spector et al. 1990, Goodman et al. 1992). Furthermore, different parameters and suitable controls may be investigated using repeated harvests in the same group of animals. The relevance of the Bone Harvest Chamber and Micromotion Chamber experiments to joint replacement is obvious: micromotion at the bone-implant interface, and the generation and biological effects of wear debris are presently thought to be two of the most important factors contributing to loosening of joint prostheses. Furthermore, a large proportion of joint replacements currently performed worldwide are noncemented. Thus, it behooves the orthopedic surgeon to understand some of the factors that may enhance or deter long-term stability of these implants.

Using the micromotion chamber, it has become apparent that one daily episode of motion of a relatively short duration may alter the degree of bone ingrowth. Factors such as the frequency, amplitude, number of daily motion periods and the interface configuration are important in determining the quality and quantity of bone ingrowth. Furthermore, the level of micromotion stimulus determined the composition of the tissue within the chamber: low levels of mechanical strain favored the formation of bone, whereas higher strains favored

the formation of fibrous tissue (the window concept). This conclusion is supported by the research of other groups (Rubin and Lanyon 1984, Goodship and Kenwright 1985, Carter 1987, Lanyon 1987, Kenwright and Goodship 1990, Carter and Giori 1991).

Our experiments with the Bone Harvest Chamber demonstrated that bone ingrowth may also be influenced by the presence of particulate debris. Using the parameters of this experiment, phagocytosable particles of bone cement, high density polyethylene and cobalt chrome alloy inhibited the ingrowth of bone into the Bone Harvest Chamber, but titanium alloy particles did not. The intrinsic properties of the particulate materials used for these experiments and/or the mode of presentation appeared to determine a characteristic tissue reaction for each material (Nagura et al. 1977, Besterman and Low 1983, Kawaguchi et al. 1986, Tabata and Ikada 1988, Perry et al. 1990). Polymer particles, such as high density polyethylene and ultra high molecular weight polyethylene are known to have a high negative surface charge compared to other materials (personal communication – Dr. Jim Davidson, Director of Orthopedic Research, Smith and Nephew, Richards, Memphis, TN, USA). Charnley (1979) also observed an aggressive foreign body response to particles of another polymer, polytetrafluorethylene, the use of which resulted in rapid clinical and radiographic prosthetic loosening. Similar problems were noted with Delrin® (Dupont) and polyester (Weber 1970, Ahnfelt et al. 1990, Ohlin 1990, Malchau et al. 1993). Materials other than polymers are currently being assessed as bearing surfaces for joint prostheses e.g. ceramics, composites and highly polished metals. The deleterious effects of particles of CoCr alloy have been previously described, and may be due to the material itself, or ionic constituents (Mital and Cohen 1968, Pappas and Cohen 1968, Rae 1975 and 1981, Howie and Vernon-Roberts 1988, Haynes et al. 1993, Maloney et al. 1993).

Despite evoking different histological reactions, both micromotion and particulate polymeric debris were noted to depress net bone formation in the micromotion chamber model. In the clinical situation, micromotion and the generation of particulate debris may work in concert. If an implant does not undergo osseointegration due to excessive micromotion, the fibrous tissue interface may provide a conduit for the migration of particles around the implant. The foreign body response due to particulate debris may then begin a vicious cycle: as bone resorption and prosthetic loosening progresses, abrasion and fretting at the interface produces increased amounts of particulate debris.

Although direct clinical application of these experiments is speculative, one may hypothesize that excessive amounts of motion and wear debris may have an adverse effect on the osseointegration of prosthetic implants, especially those employing porous ingrowth surfaces. Careful surgical technique should be employed to optimize the initial interface during prosthetic implantation. If the prosthesis is defective, scratched or mishandled during implantation, the generation of particulate debris may be accelerated. In revision cases employing a noncemented prosthesis, our findings point to the necessity of a meticulous debridement of the granuloma that often accompanies loose implants. As the interface between bone and an implant undergoes constant remodeling in response to mechanical and biological stimuli, micromotion and wear debris may have the potential to interfere with the formative processes of bone that might influence long-term prosthetic stability.

Limitations of the present research and suggestions for future work

The models

The animal models used in these experiments may have limited relevance to noncemented joint replacement in humans and specifically, bone ingrowth into porous coated prostheses in humans. The implants in rabbits are undoubtedly subjected to different loads compared to the human situation.

The strains encountered by the ingrowing tissue in the Micromotion Chamber experiments are extremely complex. Engineers at both Lund University and Stanford University have been unable to simulate the Micromotion Chamber using mathematical models. Some of the reasons for this failure include the tissue strains involved (which in certain areas are beyond the linear range), nonlinearity in the rate of application of micromotion, areas of stress concentration at corners and edges of the chamber and the existence of gaps. These features, combined with the very fine finite element mesh necessary for modeling this system, would violate the continuum assumption, which allows the application of engineering concepts for bulk materials. Future work might simulate the clinical situation more closely. A more simple design for the Micromotion Chamber, that would facilitate mathematical modeling would also be advantageous.

The chambers in our studies were fabricated from commercially pure (c.p.) titanium; this material readily osseointegrates with the surrounding bone. Although some prostheses are coated with c.p. titanium fiber mesh or beads, others are coated with sintered cobalt chrome beads that might not provide as favorable a

milieu for bone ingrowth as c.p. titanium. This point might provide a new avenue for further research.

The pore opening into the chamber was also rather large (1 mm). Several prosthesis types (e.g. the Lord Madreporic prosthesis) incorporate a macroporous design, having pores up to several millimeters in diameter. However, most current prostheses use a microporous system, with pore size ranging from 100–500 μm. The pore size appears to be an important variable affecting bone ingrowth (Bobyn et al. 1980). Future studies might examine the effects of pore size and configuration on bone ingrowth.

In the experiments using the osseointegrated Bone Harvest Chamber and Micromotion Chamber, repeated harvests were performed at three-weekly intervals. After completing one study, the animal with its chamber could enter another study, after a rest period. In ongoing studies by the authors that include some rabbits with over 20 harvests, an exhaustion effect for bone ingrowth did not occur as long as the animal is healthy. Periodic, three week "rest" periods were intermittently used to monitor this effect. It is recognized, however, that the time *in vivo* of the chamber may have affected the results. Future studies incorporating more animals may circumvent the problem of using the same animals for long periods of time.

Particulate materials

Different materials having different sizes, shapes, topographies etc. were implanted in the Bone Harvest Chamber. The cement and polyethylene particles were fabricated, the titanium alloy particles were retrieved from a hip simulator and the cobalt chrome particles were generated by milling. The aim was to use particles that were phagocytoseable (less than approximately 10–15 μm) in each experiment, however, it is likely that other properties of the particles played a role in the histological response. It is extremely difficult to generate particulate orthopedic materials that are comparable to the particles generated *in vivo*. Furthermore, it has been postulated that the most deleterious particles may be less than 1 μm in size and not discernible using the light microscope (Campbell et al 1992). Future studies, using more uniform particles or possibly utilizing retrieved particles from harvested tissues are warranted.

The concentration of particles used in this study attempted, in a simplified way, to parallel the circumstances seen clinically and histologically in aseptic loosening of joint arthroplasties (Livermore et al 1990). Current studies in progress are attemping to identify dose response curves for these different materials.

The experiments reported in this thesis document the effects of micromotion and particulate materials in the very short term (i.e. 3–6 weeks). Longer term experiments using simplified, more clinically relevant animal models would further elucidate the effects of micromotion and particulate materials on bone ingrowth and remodeling. The use of well-characterized particulate orthopedic materials, especially in the submicron range would enhance this research. Mathematical analysis of the models might prove useful in corroborating and extending the observations in the micromotion experiments.

Despite these limitations, motion and particulate materials have been shown to play important roles in the differentiation of mesenchymal tissue, using the experimental models in this thesis. These issues are of major concern to surgeons and manufacturers of orthopedic implants. Hopefully, future research will broaden our understanding of the factors that promote or impair osseointegration of orthopedic prostheses. For example, it may be possible to modulate the mechanical environment with the application of well-defined, exogenous loads to facilitate osseointegration of prostheses. New materials and bearing surfaces, with more favorable wear properties are needed to minimize the production of particles. By further understanding the biological processes associated with interfacial motion and particulate materials, other strategies may be developed to improve the longevity of orthopedic implants.

General conclusions

From these experiments, it maybe concluded that:

1) One short daily period of externally applied, low frequency micromotion may alter the degree of bone ingrowth into a 1 mm pore in a titanium implant.
2) A small mechanical stimulus may facilitate bone ingrowth, whereas a larger stimulus may inhibit bone ingrowth.
3) Factors such as the frequency, amplitude, interface configuration and number of daily motion periods are important determinants of tissue differentiation within bone.
4) Cessation of a given set of motion parameters is accompanied by tissue differentiation that reflects the new functional biomechanical environment.
5) Small phagocytosable particles of bone cement, high density polyethylene and cobalt chrome alloy, but not titanium alloy, at a concentration of 1×10^8 particles/mL are associated with a foreign body and chronic inflammatory reaction, and inhibition of bone ingrowth into a 1 mm pore in a titanium implant.
6) Despite evoking different histological reactions, both micromotion and particulate polymeric debris have an adverse effect on net bone formation in the micromotion chamber.
7) Macrophages are not dependent on T lymphocytes for their initial recruitment to the implantation of particles of high density polyethylene.

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