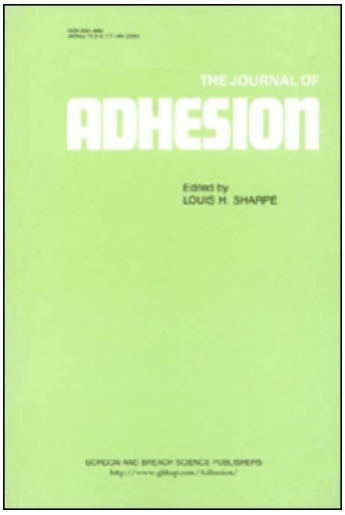

The Journal of Adhesion
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713453635

Particle-induced Phagocytic Cell Responses are Material Dependent: Foreign Body Giant Cells *Vs.* Osteoclasts from a Chick Chorioallantoic Membrane Particle-implantation Model

L. C. Carter[a]; J. M. Carter[a]; P. A. Nickerson[a]; J. R. Wright[a]; R. E. Baier[a]
[a] School of Dental Medicine and School of Medicine and Biomedical Sciences, State University of New York at Buffalo, Buffalo, NY, USA

To cite this Article Carter, L. C. , Carter, J. M. , Nickerson, P. A. , Wright, J. R. and Baier, R. E.(2000) 'Particle-induced Phagocytic Cell Responses are Material Dependent: Foreign Body Giant Cells *Vs.* Osteoclasts from a Chick Chorioallantoic Membrane Particle-implantation Model', The Journal of Adhesion, 74: 1, 53 — 77
To link to this Article: DOI: 10.1080/00218460008034524
URL: http://dx.doi.org/10.1080/00218460008034524

J. Adhesion, 2000, Vol. 74, pp. 53 - 77
Reprints available directly from the publisher
Photocopying permitted by license only

Particle-induced Phagocytic Cell Responses are Material Dependent: Foreign Body Giant Cells *Vs.* Osteoclasts from a Chick Chorioallantoic Membrane Particle-implantation Model

L. C. CARTER, J. M. CARTER, P. A. NICKERSON, J. R. WRIGHT and R. E. BAIER*

School of Dental Medicine and School of Medicine and Biomedical Sciences, State University of New York at Buffalo, Buffalo, NY 14214-3007, USA

(Received 13 March 2000; In final form 28 August 2000)

There is increasing concern about particles generated from wear-prone implants that are placed in body tissues, including artificial hip, knee, and jaw joints. Although phagocytes and foreign body giant cells are associated with inhaled or embedded particulate debris, some particles also induce bone digestion by eliciting the differentiation and proliferation of highly specialized osteoclastic cells. This report describes the differential phagocytic cellular responses to four implant-related types of ground, model wear particles in a live-egg cell-response model, as implants to the chick chorioallantoic membrane (CAM): polymethylmethacrylate (PMMA), a main constituent of some temporomandibular joint (TMJ) implants and orthopedic cements used to retain artificial hips and knees; Proplast-HA, an implantable composite of polytetrafluoroethylene (PTFE) and degradable mineral (hydroxyapatite) that has been associated with bone erosion around failed TMJ implants; talc, a nondegradable mineral sometimes found in tissues as a contaminant from talc-coated surgical gloves; and authentic bone, known to induce the formation of osteoclastic cells. Light and electron microscopy of CAM tissues harvested, sectioned and stained with special reagents for the enzymes tartrate-resistant acid phosphatase (TRAP) and tartrate-resistant adenosine triphosphatase (TrATPase), and for the osteoclast-specific antigen 121F, showed that only authentic bone and the degradable HA-rich particles induced osteoclast formation. From these results, and supporting data with polypropylene particles, it is concluded that nonbiodegradable polymer particles, alone, do not induce bone dissolution. Inert polymers do induce

*Corresponding author. Tel.: 716-829-3560, Fax: 716-835-4872, e-mail: baier@acsu.buffalo.edu

foreign body giant cells without the external mineral digestion qualities unique to osteoclasts, however. The chick embryo model system allows quick and affordable examination of material-dependent differences in phagocytic cellular responses to implant wear debris and particles from various occupational environments.

Keywords: Particles; Phagocytosis; Macrophages; Degradation; Osteoclasts; Implants

INTRODUCTION AND BACKGROUND

Particles enter the human body by many routes and from many sources. One increasing source of concern is mechanical wear debris from various alloplastic joint replacement devices not originally expected to break down under use. In certain circumstances, foreign body giant cells appear in tissues that respond to these particles. The cells then engulf and attempt to digest the particles, internally, by merging them with hydrolytic enzymes in protected membrane-bound compartments called lysosomes. Sometimes, specific bone-digesting cells – osteoclasts – also appear with unique capabilities for external digestion of large particles resistant to engulfment. It is very important to know the differences among these cell types and their inducing agents, since bone loss can lessen a patient's ability to maintain an implant securely in place.

Skeletal defects may arise from congenital anomalies, from trauma, as the sequelae of infections/inflammatory diseases or from the treatment of neoplastic processes. Regardless of their etiology, such defects frequently lead people to attempt to improve their functional and/or aesthetic status by use of a variety of autogenous and alloplastic implants. Autogenous implants, tissues taken from other places within a person's own body, are variable in terms of their availability and success rates; certainly the additional surgery needed to harvest these tissues increases morbidity. Allografts, tissues donated from other persons, raise concerns regarding infectious disease and graft/host immunocompatibility. Thus, beginning in the 1960s, biomedical engineers and surgeons increasingly turned to a host of alloplastic materials both metallic (*e.g.*, cobalt-chromium-molybdenum alloy, Vitallium [Howmedica, West Berlin, NJ]) and polymeric (*e.g.*, medical grade silicone rubber, Silastic [Dow Corning Corporation, Midland, MI]) for the fabrication of substitute human joints.

In cases of hip, knee, and temporomandibular joint (TMJ) replacements, cobalt-chromium alloy is sometimes cemented in place with polymethylmethacrylate (PMMA) "bone cement", or PMMA is used for the actual articulating surfaces, that could spall fugitive particles and produce tissue trauma. We showed, earlier, how physical breakdown and particle production from Silastic used in TMJ interpositional implants triggered local foreign body giant cell responses accompanied by migration of particles to regional lymph nodes [1].

Other contemporary alloplastic implants incorporated a series of porous composite materials called Proplast (Vitek, Inc., Houston, TX) [2]. Based on mixtures of polytetrafluoroethylene (PTFE) with carbon or alumina (Proplast II), and later with hydroxyapatite (Proplast-HA), these composites were used for facial augmentation procedures as well as TMJ implants. Many investigators have since reported that particles shed from Proplast composites could elicit significant foreign body inflammatory giant cell responses which, in the case of Proplast HA, also were associated with severe osseous resorption [2–7].

The unfortunate clinical results were significant posterior migration of chin implants through bone, erosion of condyles and articular eminences and, in some cases, perforation of the middle cranial fossa and dura mater [3–5], exposing the brain. Marked foreign body giant cell reactions were reported to occur even in sites such as the middle ear, where tissue motion adjacent to the implant was not a plausible explanation for effects seen [6]. These particle-induced responses were so universal and severe that Proplast interpositional TMJ implants were withdrawn from the market in 1988, with the Food and Drug Administration (FDA) warning that total TMJ devices could present similar risks [7].

Today, thousands of patients retain devices containing bone cement in orthopedic prostheses and PMMA articulations in TMJ implants, and Proplast-HA in facial augmentations or TMJ reconstructions. It is important that the basic biologic response to particles of these materials be ascertained. This report focuses on the different abilities of PMMA particles and Proplast-HA particles to induce foreign body giant cells with osteoclast-like (osteolytic, bone dissolving) properties, using the embryonated chicken egg chorioallantoic membrane model

developed by Krukowski and Kahn [8]. Similarly-sized particles of authentic bone (from chick tibiae) were used as "digestable" particle controls, and talc particles (as used to lubricate surgical gloves, and often inadvertently entering into wound sites) were used as non-degradable mineral controls. These results are extracted from a comprehensive series of particle studies in the same model, in which confirming data were obtained with particles of non-degradable polypropylene (suture material) and differentially degradable compositions of polyglycolic acid and polylactic acid [9].

MATERIALS AND METHODS

NIH guidelines for the care and use of laboratory animals (NIH publication #85-23 Rev. 1985) were observed during the conduct of this study.

Chorioallantoic Membrane Implantation

Chick tibiae, Proplast-HA, polymethylmethacrylate (PMMA) and talc were comminuted by cryogenic milling and sieved to the 75–150 μm particle size range. Talc was received as a nominally 40 μm particle size powder with many multi-particle agglomerates of larger size and was treated similarly to ensure uniformity of handling of all specimens. Two-milligram samples were sterilized by exposure to gamma irradiation at a delivered minimum dose of 3.51 Mrad and a delivered maximum dose of 4.07 Mrad for 275 minutes (Isomedix, Whippany, NJ). On day 5 of their incubation, 48 embryonated white Leghorn chicken eggs were windowed according to the methods described by Krukowski and Kahn [8]. After an additional 4 days, the chorioallantoic membranes (CAMs) were implanted with one of the particulate samples. After 9 days of implantation (day 18 of incubation), the induced cell plaques were harvested and analyzed by light microscopy, histochemistry, immunocytochemistry and electron microscopy, and were fragment-cultured using a bone slice resorption assay [10–12]. In addition, tibial medullary bone from 3 chick hatchlings, maintained on a low calcium diet for 4 weeks, served as an osteoclast-positive control for these assays.

Light Microscopy

Cell plaques were fixed in 10% neutral buffered formalin, dehydrated in a graded series of alcohols and embedded in paraffin. Sections were cut at 5 μm and stained with hematoxylin and eosin.

Histochemistry

Cell plaques destined for histochemistry were fixed in 2% glutaraldehyde in 0.1 M sodium cacodylate buffer with 7% sucrose at pH 7.4, embedded in JB-4 resin (Polysciences, Warrington, PA), sectioned at 3 μm and stained for either tartrate-resistant acid phosphatase (TRAP) or tartrate-resistant adenosine triphosphatasae (TrATPase) [13, 14]. Sections incubated without substrate or in the presence of sodium-ortho-vanadate served as negative controls. Sections of chicken spleen served as a positive control for TRAP and as a negative control for TrATPase reactivity. Reagents for histochemistry were purchased from Sigma Chemical Co. (St. Louis, MO).

Immunocytochemistry

Cell plaques for study by immunocytochemistry were fixed in periodate-lysine-paraformaldehyde and cryosectioned at 5 μm [15]. Using a biotinylated streptavidin peroxidase-antiperoxidase staining protocol, cryosections were incubated with primary monoclonal antibody 121F raised against chicken osteoclasts at a dilution of 1 : 500 and secondary antibody goat-antimouse IgG (1 : 250) [15]. Sections incubated in the absence of primary antibody served as negative controls. Reagents for immunocytochemistry and cell culture were obtained from GIBCO/BRL Life Technologies Inc. (Bethesda, MD).

Transmission Electron Microscopy

CAM plaques were fixed in 2% glutaraldehyde in 0.1 M sodium cacodylate buffer at pH 7.4, post-fixed in 1% osmium tetroxide and embedded in Epon-Araldite (Fullam, Inc., Schenectady, NY). Thin sections (0.05 μm) were cut with a diamond knife, mounted on copper grids, and stained with aqueous uranyl acetate and lead citrate.

Bone Slice Resorption Assay

For the bone slice resorption assay, additional CAM cell plaques or medullary bone from hypocalcemic chicks were fragment-cultured on devitalized bovine cortical bone wafers in α-MEM in HEPES buffer supplemented with penicillin and streptomycin, 10% NCS (calf serum)

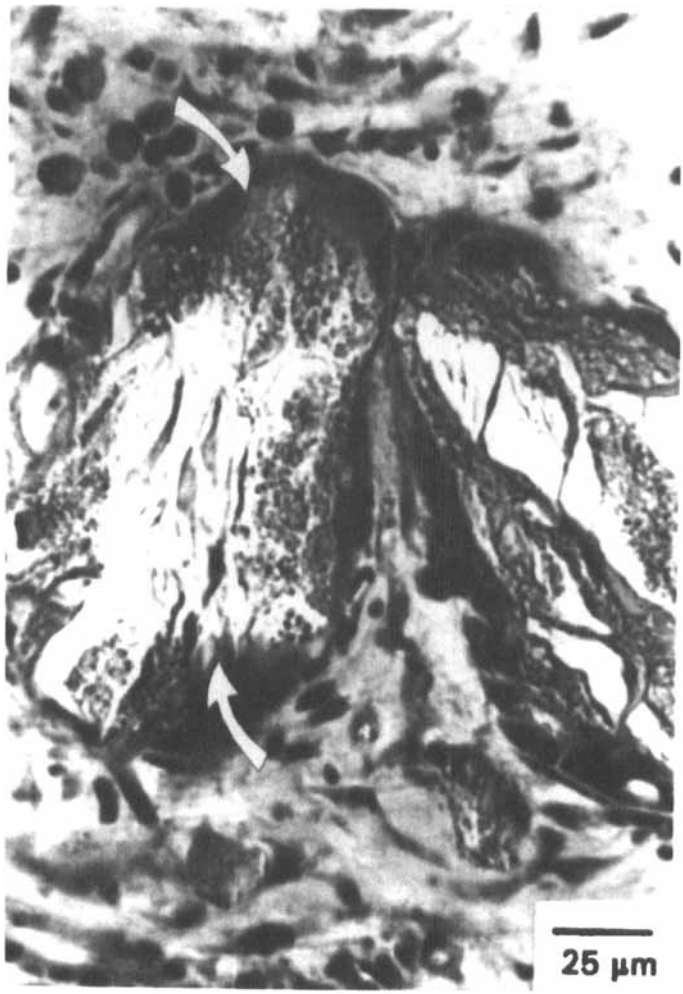

FIGURE 1a Amphophilic transition zones between giant cells and Proplast-HA are viewed (arrows) along with Pseudopodial extension of cytoplasm into the composite. Hematoxylin and eosin staining used.

at 37°C in a humidified atmosphere of 95% air and 5% CO_2 [12]. After incubation for 24 or 48 hours, wafers were fixed in 2% glutaraldehyde in 0.1 M sodium cacodylate buffer at pH 7.3, stained with 1% toluidine blue in 0.5% disodium tetraborate and examined by brightfield transmitted light microscopy for osteoclast assessment. On an additional set of wafers, cells were stripped by immersion in 5.25% NaOCl for 25 minutes followed by brief ultrasonication. These wafers then were sputter-coated with gold under an argon atmosphere for 1.5 minutes at 20 mA in a vacuum evaporator. Wafers were examined for the presence of resorption pits using darkfield reflected

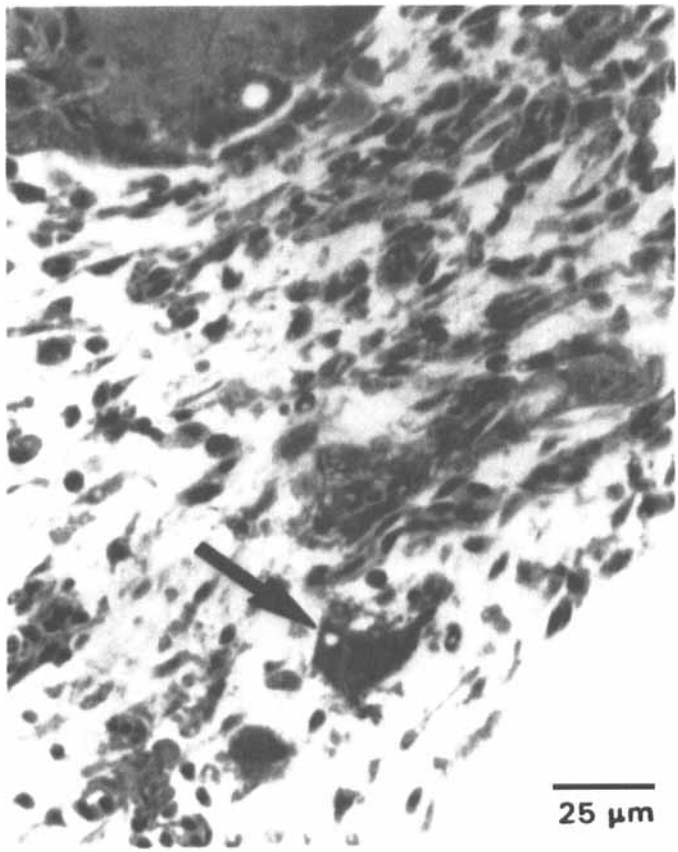

FIGURE 1b Photomicrograph reveals phagocytosis of talc particles by several polykarya including one that already harbors internalized talc (arrow). Hematoxylin and eosin staining used.

light microscopy [16]. Wafers similarly cultured in the absence of cells served as negative controls.

RESULTS

Chick Embryo Plaque Qualities

CAM foreign body giant cells induced by implantation of Proplast-HA showed an amphophilic zone of interdigitation between the cytoplasm

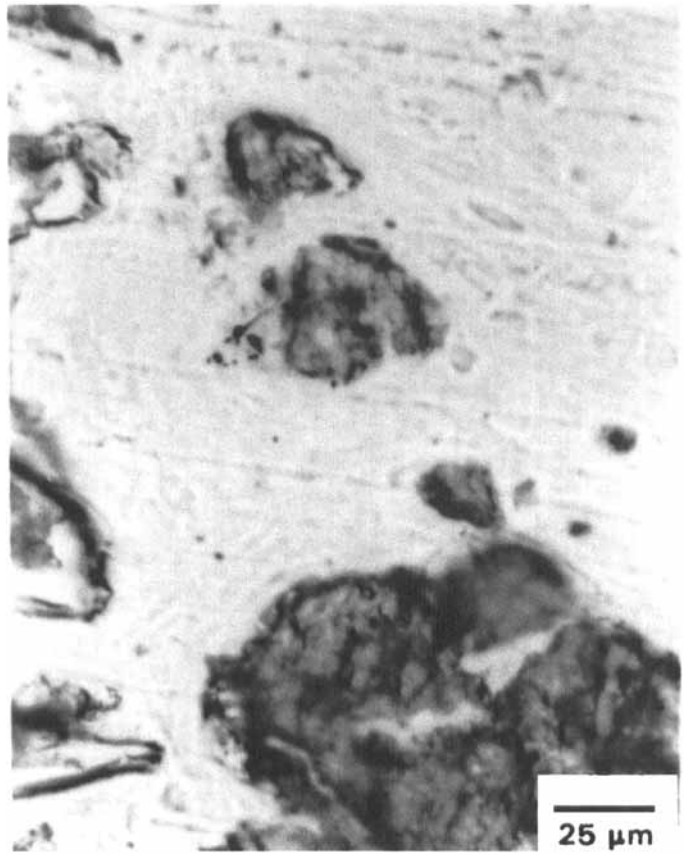

FIGURE 2 A fine, dark, granular precipitate is viewed in and vicinal to polykarya induced by implantation of particulate Proplast-HA. TrATPase, no counterstain.

and the composite, by light microscopy (Fig. 1a). In this zone, both the normal eosinophilic character of the cell cytoplasm and the normal grayish granular refractile nature of the particulate Proplast-HA material were lost. The cells' cytoplasm areas appeared foamy. Although implantation of PMMA and talc onto the CAM also elicited the formation of numerous foreign body giant cells, the appearance of the interfacial zone between particles and cells was quite different. Instead of an amphophilic transition zone, the interfaces between the PMMA or talc particles and the cells were crisp and distinct. Many of the active cells were photographed as they engaged in engulfment, phagocytosis and internalization of the PMMA or talc particles (Fig. 1b).

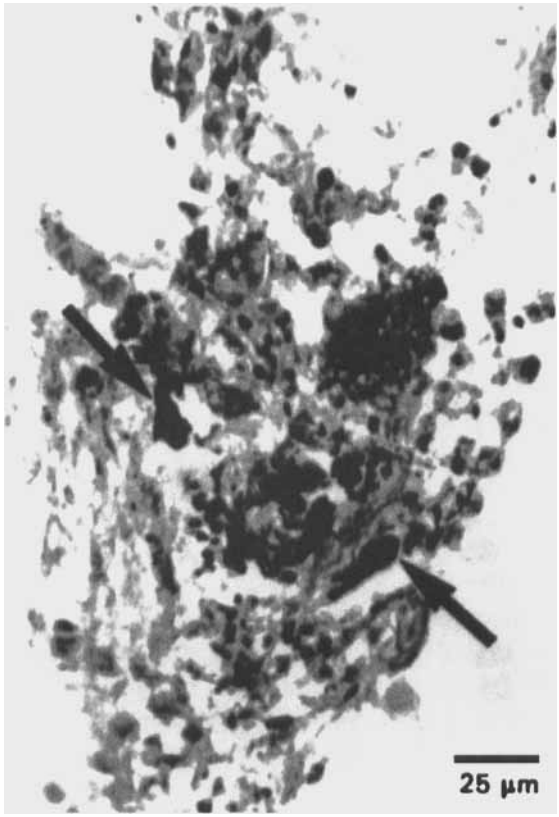

FIGURE 3a Moderate reactivity for the 121F antigen is observed in multinucleated and some mononuclear cells associated with implanted particulate Proplast-HA (arrows).

Special Stain Outcomes

Endosteal osteoclasts and many mononuclear cells from tibial medullary bone of calcium-deficient chick hatchlings displayed strong staining for TRAP, visualized as a granular maroon-colored reaction product. Staining of these cells for TrATPase, viewed as a dark brown stain with a black precipitate, also was intense, except that fewer mononuclear cells reacted, and the background osseous staining was less intense for TrATPase than for TRAP. Osteoclasts, induced after implantation of particulated tibiae on CAMs, displayed a similar

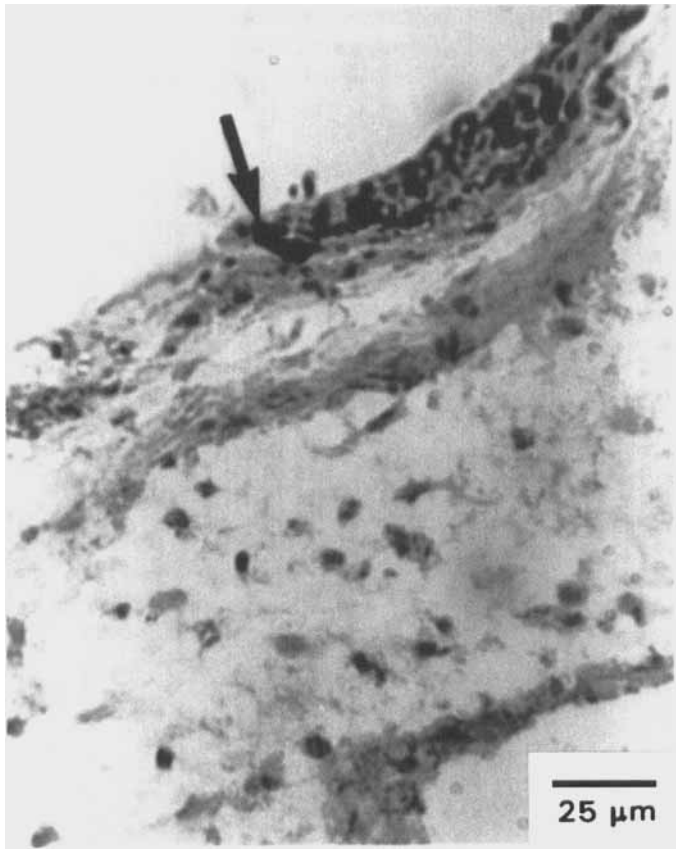

FIGURE 3b Biotinylated streptavidin immunoperoxidase stain, 121F monoclonal antibody, hematoxylin counterstain.

pattern of staining. Foreign body giant cells induced by implantation of Proplast-HA also stained intensely for both TRAP and TrATPase (Fig. 2).

On the other hand, PMMA- and talc-induced foreign body giant cells showed only mild to moderate TRAP reactivity and a total dearth of reactivity for TrATPase.

Sections of spleen displayed focal TRAP-positive cells which were distributed throughout the marginal zones of the tissue, but there was a complete absence of reactivity for TrATPase. Incubation of

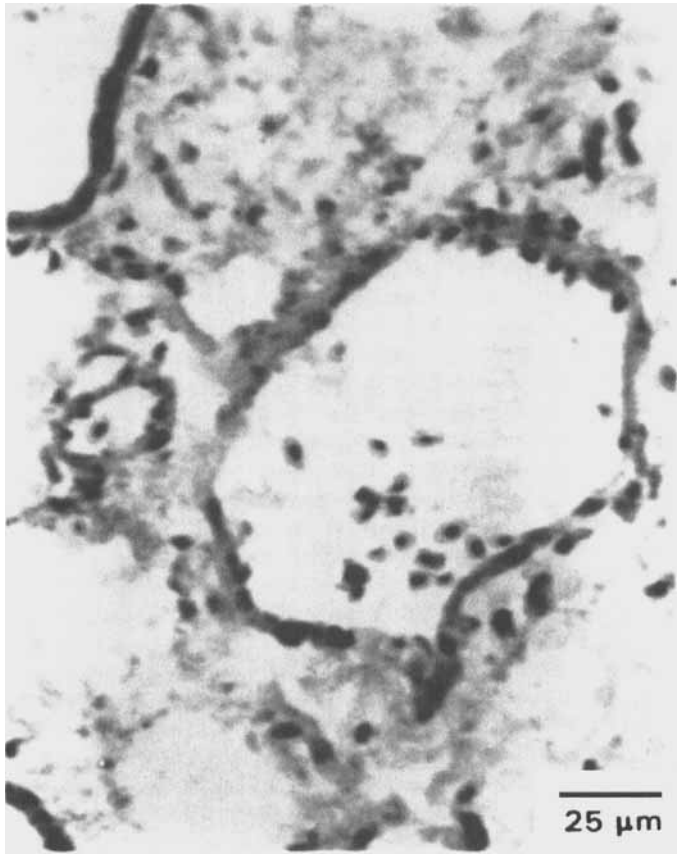

FIGURE 3c CAM polykarya induced by implantation of polymethylmethacrylate fail to react with the 121F monoclonal antibody. Biotinylated streptavidin immunoperoxidase stain, 121F monoclonal antibody, hematoxylin counterstain.

specimens with sodium-ortho-vanadate or in the absence of the particle substrates, completely extinguished the TRAP or TrATPase activities.

Antigen-antibody Reactions

Strong reactivity directed against the 121F antigen was visualized as a granular brown cytoplasmic stain in osteoclasts and mononuclear cells of hypocalcemic chick medullary bone. Multi- and

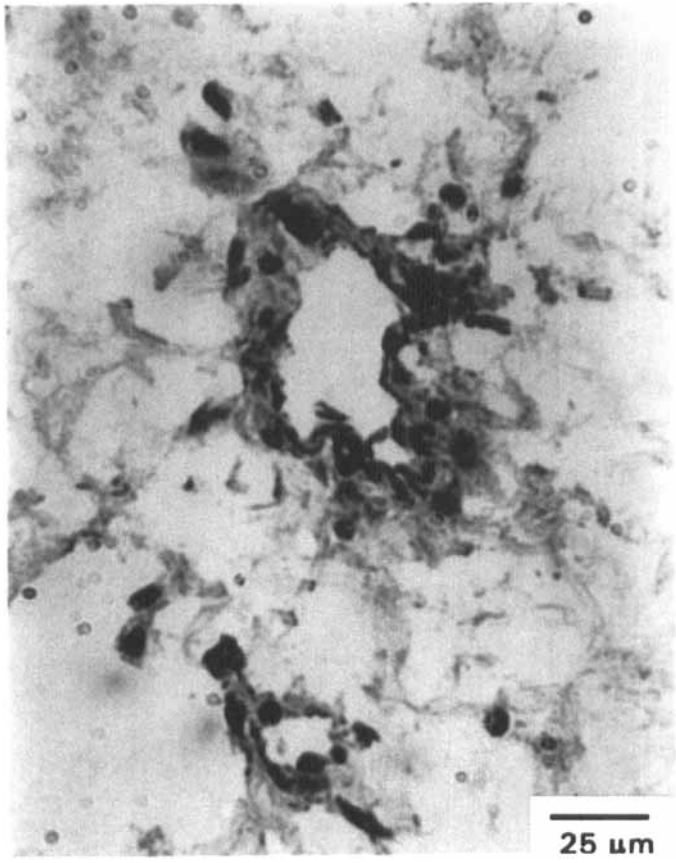

FIGURE 3d CAM polykarya induced by implantation of talc fail to react with the 121F monoclonal antibody. Biotinylated streptavidin immunoperoxidase stain, 121F monoclonal antibody, hematoxylin counterstain.

mononuclear CAM cells intimately associated with particulate chick tibiae or with Proplast-HA displayed a moderately intense positivity with the 121F monoclonal antibody (Figs. 3a, b). No reactivity was observed in CAM foreign body giant cells induced by implantation of PMMA or talc (Figs. 3c, d). Negative controls incubated in the absence of primary antibody failed to demonstrate any reactivity.

FIGURE 4a Well-developed ruffled borders are viewed in foreign body giant cells raised in response to CAM implantation of Proplast-HA particles. Many polyribosomes and mitochondria (M) as well as numerous thickened membrane specializations (arrowheads) are viewed in this electron micrograph. Extracellular channels of the ruffled border contain partially degraded substrate.

Electron Microscopic Features

Transmission electron microscopy revealed that foreign body giant cells associated with either CAM-implanted particulated chick tibiae or Proplast-HA displayed membrane ruffling against those implanted materials, this being better developed in the bone-particle specimens than in the PTFE-HA composite specimens. In both cases, partially degraded particulate matter was present within the extracellular channels of the foreign body giant cells' ruffled borders (Fig. 4a).

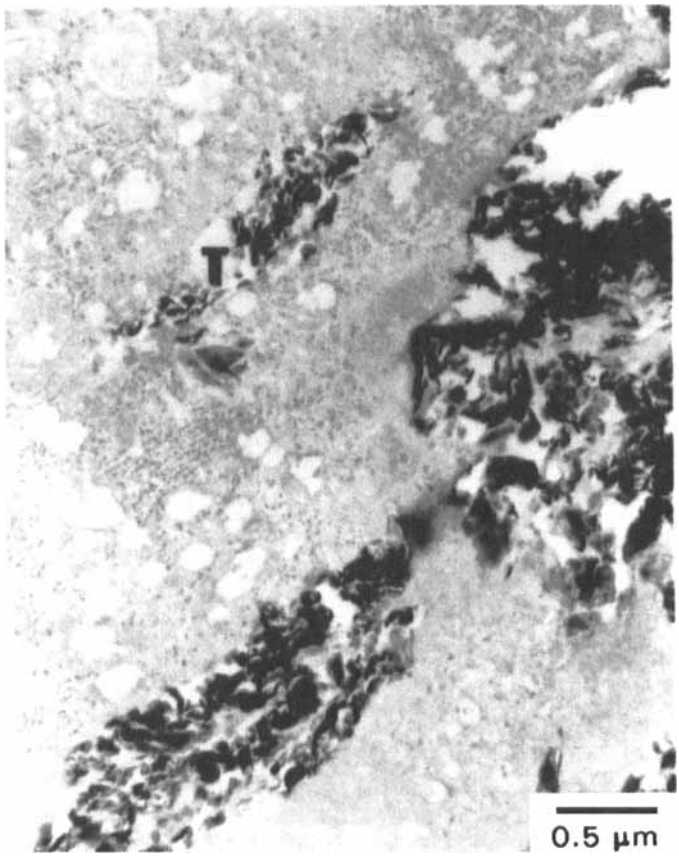

FIGURE 4b Internalized (phagocytosed) talc particles (T), and extensive rough endoplasmic reticulum are viewed within this polykaryon. Note lack of ruffled border adjacent to particulate material.

Extensive cytoplasmic complexity characterized by an abundance of mitochondria, polysomes, vacuoles and vesicles typified foreign body giant cells from both the bone particle specimens and Proplast-HA specimens. Numerous thickened membrane specializations were viewed along the degradable particles in contact with foreign body giant cells' ruffled border membranes. On the other hand, non-degradable particles induced foreign body giant cells that did not have ruffled borders. Such cells were found in close contact with talc and PMMA, and even phagocytosed some small fragments of these materials (Fig. 4b), but there was no evidence for either external

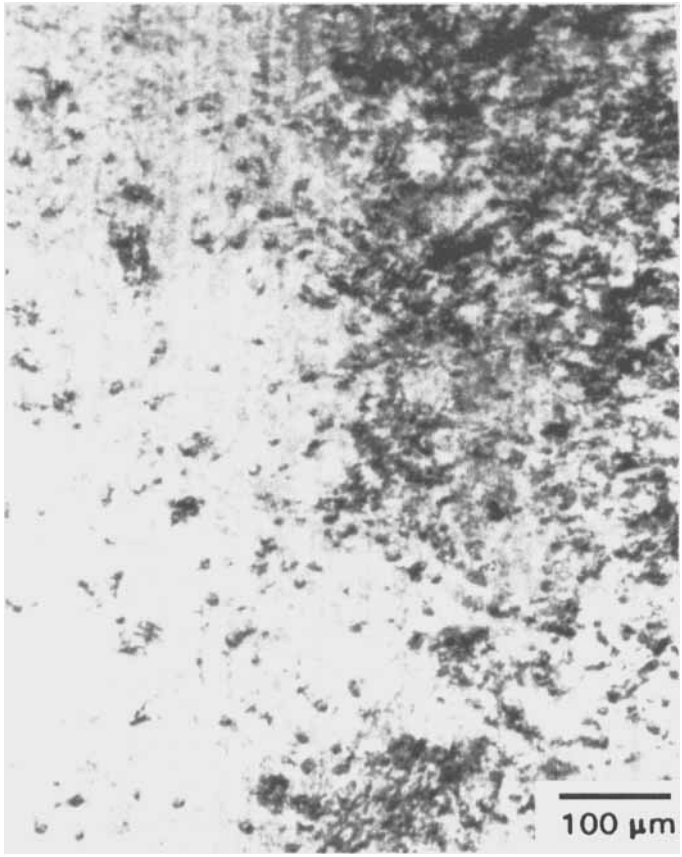

FIGURE 5a After 48 hours in culture, a bone wafer is densely populated by adherent osteoclasts from hypocalcemic chick tibiae. Toluidine blue, reflected light.

or internal material digestion. While plasmalemmal (membrane) interdigitation was viewed on the dorsolateral portions of these foreign body giant cells, raised against nondegradable particles, membrane regions in contact with the particulate PMMA and mica implants were smooth. Rough endoplasmic reticulum was quite prominent within these same cells, but overall cytoplasmic complexity was reduced in comparison with the tibiae- and Proplast-HA-induced cells. No membrane specializations of any type were found for the foreign body giant cells around the talc and PMMA particles.

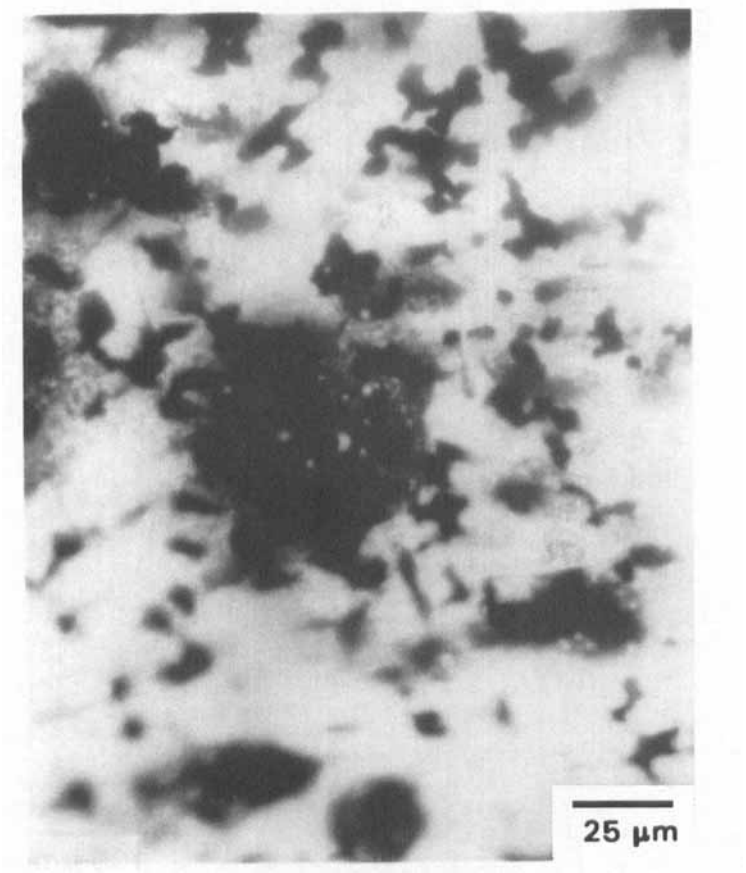

FIGURE 5b After 24 hours of culture, foreign body giant cells from CAM implanted with Proplast-HA are characterized by large, well-spread cytoplasmic skirts and profound vacuolization. Toluidine blue, transmitted light.

Bone Digestion Differences

Bone wafers, fragment-cultured with authentic osteoclasts from hypocalcemic chicks or with chick tibiae-induced CAM foreign body giant cells, displayed numerous adherent cell clusters at 24 hours which developed into a thick confluent cellular carpet by 48 hours. Adherent cells were flat, well spread and displayed prominent vacuolization. Underlying wafers became deeply excavated (dissolved or digested) and displayed numerous overlapping resorption pits and

FIGURE 5c After 48 hours in culture, the population of Proplast-HA-induced CAM foreign body giant cells has greatly expanded. Confluence of resorption pits and tracks is viewed across the bone wafer. Toluidine blue, reflected light.

tracks. The excavations were most prominent in the case of wafers cultured with marrow cells from the chick hatchlings maintained on a low calcium diet (Fig. 5a). CAM multinucleated foreign body giant cells raised by implantation of Proplast-HA also displayed large, well-spread cytoplasmic skirts and profound vacuolization (Fig. 5b). After 48 hours in culture on bone wafers, extensive confluence of excavation lacunae was observed (Fig. 5c). No excavations were observed on bone wafers cultured in the absence of cells or with talc- or PMMA-induced CAM plaques (Fig. 5d).

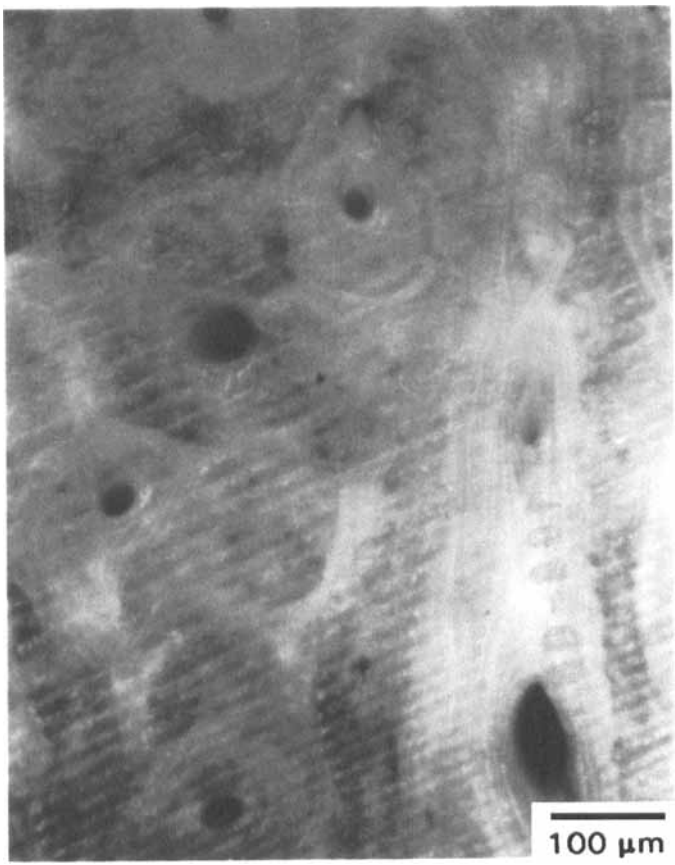

FIGURE 5d No excavations are apparent on this bone wafer cultured with CAM foreign body giant cells elicited by talc. Wafer stripped of cells after 24 hours in culture, toluidine blue, sputter-coated, reflected light.

DISCUSSION

Inequalities of Implant Materials

The question to be resolved is this: Do polymer or mineral particles have differential effects on the bone-dissolving qualities of the body's reactive phagocytes? Proplast-HA is a composite of an inert perfluorinated polymer (Teflon [DuPont, Wilmington, DE]), and acid-soluble hydroxyapatite (calcium phosphate). Perfluorocarbon polymers, as a group, display useful chemical and thermal properties as well as a low modulus of elasticity, which initially piqued the interest of the implant community [2]. Early Proplast materials, as porous composites of polytetrafluoroethylene (PTFE) with carbon or alumina, were used beginning in the mid-1960's as fillers in many non-load-bearing areas of the body [17]. Proplast interpositional implants, developed as alloplastic replacements for human TMJ discs, were inserted post-discectomy to maintain or restore vertical dimension, to avoid an anterior open bite or functional mandibular deviation, and to provide a barrier to the formation of adhesions, and ankylosis [3, 5, 18]. In the early 1980's, laminates were made to Teflon sheet, using Proplast with an admixture of either vitreous carbon fibers (Proplast I) or aluminum oxide whiskers derived from sapphire crystals (Proplast II). The porous Proplast portion was placed against the glenoid fossa to elicit bone ingrowth, while the smooth Teflon face of the laminate apposed the articular surface of the condyle [19, 20].

Particle generation occurred, but bone digestion was not reported. Later, when Proplast-HA was surface-laminated to an ultra-high-molecular-weight polyethylene articular surface, and mated against a metallic (CoCrMo) condylar head, particulate Proplast debris became associated with significant osteolysis as well as the prior-observed inflammatory responses [3, 5, 18].

Clinical Concerns

Kent noted that the great interest in use of these alloplastic devices during the years 1982–1986 slowed with an increasing number of animal and human studies reporting bone resorption, hypomobility, malocclusion and pain associated with the foreign body giant cell

responses against microscopic wear debris from these newer implants [21]. Similar deleterious effects were reported, with symptoms of pain, burning sensation in the joint, crepitance, limited range of excursion, pressure around the eyes and teeth, myalgia, headache, infection, preauricular swelling, acute noninfectious lymphadenopathy and generalized weakness and malaise [5, 22–24]. Radiographic evidence confirmed the morbid sequelae: severe erosion of facial bones or destruction of condyle/fossa/base of skull complex, implant migration and fragmentation and perforation into the middle fossa with dural violation accompanied by cerebrospinal fluid leakage [3, 5, 25, 26]. How much of this problem is caused by inert wear particles of the PTFE polymer, and how much by the degradable HA mineral particles of Proplast-HA?

Reactions to Inert Materials

In 1962, Charnley abandoned the use of PTFE acetabular cups for artificial hips because particulate wear debris produced severe foreign body reactions, resulting in the production of granulomatous tissues and osseous erosion [27]. Since bone wear particles or surgical debris from bone trimming might have placed osteoclast-inducing bone particles into the same environment as the PTFE wear debris, creating a mixture not unlike that of the Proplast-HA product, no clear separation of these effects is available from the historical clinical data. Rooney and co-workers noted that, despite the general biocompatibility of PTFE, a foreign body giant cell response always is elicited and this is irrespective of the load or site of implantation, citing similar reactions in response to periurethral injections, orbital implants, vascular grafts, ossicular implants, laryngeal implants and joint replacements [28]. Spector and colleagues raised the possibility that, although porous Proplast with carbon fiber was designed to be osteoinductive, an overwhelming foreign body giant cell response played a role in inhibiting bone formation against and into the implants [29], but without notable bone dissolution.

Other polymers may be more benign than PTFE. Even in Proplast-HA systems, where 21 of 118 total joints with polyethylene articulating surfaces had been removed, there was no incidence of failure due to polyethyelene wear debris [20, 30].

Reactions to Degradable Materials

Spagnoli and Kent advised scrupulous removal of all Proplast-HA and granulomatous tissue during implant retrievals because the abundant particle debris present would continue to incite cells which elaborated inflammatory mediators IL-1 [interleukin-1] and PGE_2 [prostaglandin E_2] to stimulate bone resorption [22], in addition to the osteoclast induction noted in the present study. Carter reported that degradable poly(glycolic acid) and poly(lactic acid) particles also induced the production of foreign body giant cells with osteoclastic phenotypes [9].

Implications for Biomaterials Selection

Most reports of implant-generated particles also cite the development of an exuberant foreign body giant cell reaction, but foreign body giant cells and osteoclasts are virtually indistinguishable on light microscopic examination. The results reported here from a battery of assays, taken together, are the first to reveal distinctly different distributions of such reactive cells in relation to specific material/particle compositions. Multinuclearity and TRAP positivity proved not to be sufficiently reliable markers for the more bone-damaging osteoclast phenotype. TRAP is also expressed in splenic macrophages, pulmonary alveolar macrophages, and in multinucleated giant cells in a variety of pathologic states. Thus, although TRAP represents a marker for macrophage activation, it is not sufficiently specific – alone – to confirm cellular differentiation along the osteoclastic lineage. Mononuclear phagocytes can express the TRAP enzyme and fuse into multinuclear giant cells (polykaryons) without any apparent relationship to osteoclast differentiation.

Biomaterials choice requires that the excessive tissue damage from osteoclasts be avoided, this damage relating to their ability to degrade the biomaterials externally and to dissolve large-surface-area plates of bone. Osteoclasts degrade other objects too large to be engulfed, by exuding acids and enzymes into external pockets they seal against the material to be digested. Osteoclasts contain abundant lysosomal acid hydrolases, including TRAP, which are actively secreted into external resorption lacunae, representing the

physiologic and chemical counterpart of the internal digestive lysosomes of all phagocytic cells [31]. Ascribing an osteoclastic identity to a cell type induced by any particular biomaterial requires additional morphologic evidence as well as demonstration of the appropriate antigens and ability to function in an external resorptive capacity.

TrATPase is an excellent additional indicator, as a unique member of the TRAP family of acid hydrolase isoenzymes which is secreted vectorially into the external resorption compartment and is expressed only in osteoclast ontogeny [32]. The inability of macrophagic TRAP enzymes to hydrolyze ATP as substrate renders TrATPase a more selective marker than TRAP for osteoclast identification. For example, while splenic macrophages stain for TRAP, they do not express TrATPase acitivity [33], a finding that was confirmed in our study.

Free Radical Reactions

The 121F antigen is related to the superoxide (anion, free radical) dismutase molecule and also is associated with the extracellular breakdown of resorbable or particulate material [15]. Hence, 121F antigen expression also can predict or confirm the cell's functional ability to excavate bone, and implicate further reactions of hydrogen peroxide which is generated by the superoxide dismutase reaction with the superoxide anion. Critical observations were made in our cell culture assays whereby isolated cells were allowed to excavate resorption pits (lacunae) on mineralized substrates. This test of functional activity already has served as a catalyst for major advances in osteoclast biology research [34], and will be a valuable method for future studies of material-composition-dependent differentiation of human phagocytic cells. The technique used here, whereby bone wafers were sputter-coated to reveal the topography of the resorbed surface when examined under dark field reflected light microscopy [16], provides the ability to detect recently active excavations while the cells remain *in situ*.

Production and harvesting of sufficient reactive phagocytic cells is a major requirement to further advance understanding of the body's response to small particle "implants".

Extensions to "Tissue Engineering"

In the chick CAM model, the implantation of particulate matter initiates a cascade of predictable events culminating in the induction of numerous foreign body giant cells. This implant system represents an affordable *in vivo* model for investigation of the inductive specificity of various biomaterials for polykaryon ontogeny and ectopic osteoclast differentiation. Only implant materials capable of being degraded and resorbed extracellularly (Proplast-HA, poly(glycolic acid), and poly (lactic acid), as well as bone chips) induce cellular fusion and differentiation events indicative of osteoclast production in the CAM model [9]. Webber and colleagues reported a similar pathway for resorbable carbonate- and barium sulfate-induction of giant cells [15]. "Tissue Engineering" anticipates the exclusive use of resorbable, degradable scaffolds for cellular regeneration around high-surface-to-volume implants [35, 36] so osteoclast-like polykaryon reactions should be anticipated.

On the other hand, (1) development of TrATPase positivity, (2) expression of the 121F antigen and (3) ability to produce resorption pits on bone wafers did *not* occur with CAM foreign body giant cells elicited by nonresorbable substrata such as PMMA, polypropylene, or talc [9]. Webber and co-workers earlier reported a lack of 121F antigen expression on giant cells elicited by crosslinked Sepharose beads, PMMA and mica [15]. Osteoclast-specific features might provide morphologic and functional confirmation of an "engineered" tissue's ability to degrade its extracellular scaffold.

CONCLUSIONS

On the chick CAM, Proplast-HA particles induced multinucleated giant cells that were osteoclast-like by both morphologic and functional criteria. Osteoclast induction by degradable implant substances is the likely mechanism of severe bone erosion reported in patients with some types of implant materials, and not others, in spite of apparently equally severe inflammatory responses to particulate wear debris. Implant failures for inert materials in the TMJ, including implants fabricated from Silastic, did not lead to the

severe osseous destruction reported with Proplast-HA [30, 37]. This conclusion is supported by noting that Proplast-HA uniquely contained a degradable mineral component, calcium phosphate, rather than the inert silica filler of Silastic, or the inert carbon or alumina components of Proplast I and II. Furthermore, bone erosion also was seen adjacent to facial augmentation prostheses fabricated of Proplast-HA where the implants were not under obvious loads. Since bone erosion was not reported for the earlier Proplast versions, and PTFE polymer (Teflon) was the common material in all three Proplast products, the degradable HA mineral particles were the most probable osteoclast-inducing factor.

Acknowledgments

We extend special thanks to Dr. Philip Osdoby of Washington University (St. Louis, MO) for providing the 121F monoclonal antibody and, along with Dr. C. Z. Liu of the University of Washington (Seattle, WA), for kind advice and continuing research guidance. We gratefully acknowledge Janet Gorfien, Maria Kozak, and Sandra Mendel for expert technical assistance and Elisabeth Lawson for preparation of the micrographs. This study was supported in part by an American Fellowship from the American Association of University Women and the Mark Diamond Research Fund (GSA) of the State University of New York at Buffalo.

References

[1] Hartman (Carter), L., Bessette, R., Baier, R., Meyer, A. and Wirth, J., *J. Biomed. Mater. Res.* **22**, 475 (1988).
[2] Homsy, C., In: *Biocompatibility of clinical implant materials* (CRC Press, Inc. Boca Raton, 1982), pp. 59–77.
[3] Berarducci, J., Thompson, D. and Scheffer, R., *J. Oral Maxillofac. Surg.* **48**, 496 (1990).
[4] Stambaugh, K., *Arch. Otolaryngol. Head Neck Surg.* **118**, 682 (1992).
[5] Chuong, R. and Piper, M., *Oral Surg., Oral Med., Oral Pathol.* **74**, 422 (1992).
[6] Brennan, G., Kerr, A. and Smyth, G., *Clin. Otolaryngol.* **9**, 229 (1984).
[7] AAOMS: TMJ Implant Advisory. Rosemont, IL., AAOMS, August, 1992.
[8] Krukowski, M. and Kahn, A., *Calcif. Tissue Int.* **34**, 474 (1982).
[9] Carter, L. C., *Analysis of the Cellular Healing Response of the Chick Chorioallantoic Membrane to Implanted Poly(Glycolic Acid)*, Doctoral Dissertation, State University of New York at Buffalo (1993).
[10] Arnett, T. and Dempster, D., *Endocrinology* **119**, 119 (1986).

[11] Arnett, T. and Dempster, D., *Endocrinology* **120**, 602 (1987).
[12] Dempster, D., Murrills, R., Horbert, W. and Arnett, T., *J. Bone Miner. Res.* **2**, 443 (1987).
[13] Barka, T. and Anderson, P., *J. Histochem. Cytochem.* **10**, 741 (1962).
[14] Andersson, G. and Marks, S., *J. Histochem. Cytochem.* **37**, 115 (1989).
[15] Webber, D., Osdoby, P., Hauschka, P. and Krukowski, M., *J. Bone Miner. Res.* **5**, 401 (1990).
[16] Walsh, C., Beresford, J., Birch, M., Boothroyd, B. and Gallagher, J., *J. Bone Miner. Res.* **6**, 661 (1991).
[17] Wagner, J. and Mosby, E., *J. Oral Maxillofac. Surg.* **48**, 1140 (1990).
[18] Gallagher, D. and Wolford, L., *J. Oral Maxillofac. Surg.* **40**, 672 (1982).
[19] Florine, B., Gatto, D., Wade, M. and Waite, D., *J. Oral Maxillofac. Surg.* **46**, 183 (1988).
[20] Kent, J., Block, M., Halpern, J. and Fontenot, M., *J. Oral Maxillofac. Surg.* **51**, 408 (1993).
[21] Kent, J., *J. Oral Maxillofac. Surg.* **49**, 443 (1991).
[22] Spagnoli, D. and Kent, J., *Oral Surg., Oral Med., Oral Pathol.* **74**, 411 (1992).
[23] Lagrotteria, L., Scapino, R., Granston, R. and Felgenhauer, D., *J. Craniomandib. Pract.* **4**, 172 (1986).
[24] Feinerman, D. and Peicuch, J., *Int. J. Oral Maxillofac. Surg.* **22**, 11 (1993).
[25] Valentine, J., Reiman, B., Nat, R., Beuttenmuller, E. and Donovan, M., *J. Oral Maxillofac. Surg.* **47**, 689 (1989).
[26] Heffez, L., Mafee, M., Rosenberg, H. and Langer, B., *J. Oral Maxillofac. Surg.* **45**, 657 (1987).
[27] Charnley, J., *Fed. Proc.* **25**, 1079 (1962).
[28] Rooney, T., Haug, R., Toor, A. and Inaresano, A., *J. Oral Maxillofac. Surg.* **46**, 240 (1988).
[29] Spector, M., Harmon, S. and Kreutner, A., *J. Biomed. Mater. Res.* **13**, 677 (1979).
[30] Bronstein, S., *Oral Surg., Oral Med., Oral Pathol.* **64**, 135 (1987).
[31] Andersson, G., Ek-Rylander, B. and Minkin, C., "Acid phosphatases". In: Rifkin, B. and Gay, C. (Eds.), *Biology and Physiology of the Osteoclast* (CRC Press, Boca Raton, 1992), pp. 55–73.
[32] Flanagan, A. and Chambers, T., *J. Pathol.* **159**, 53 (1989).
[33] Linduger, A., Mackay, C., Ek-Rylander, B., Andersson, G. and Marks, S., *Bone and Mineral* **10**, 109 (1990).
[34] Boyde, A., Ali, N. and Jones, S., *Br. Dent. J.* **56**, 216 (1985).
[35] Langer, R., *Accounts Chem. Res.* **33**, 94 (2000).
[36] Langer, R. and Vacanti, J. P., *Science* **260**, 920 (1993).
[37] Timmis, D., Aragon, S., Van Sickels, J. and Aufdemorte, T., *J. Oral Maxillofac. Surg.* **44**, 541 (1986).