Scanning Microscopy

Volume 4 | Number 2 Article 9

6-15-1990

Scanning and Transmission Electron Microscopy, and Electron Probe Analysis of the Interface Between Implants and Host Bone

G. Daculsi
Laboratoire de Recherche sur les Tissus Calcifies et les Biomateriaux Faculte de Chirurgie Dentaire

R. Z. LeGeros
New York University College of Dentistry

C. Deudon
Groupement D'Interet Scientifique GIS Biomateriaux

Scanning Microscopy, Vol. 4, No. 2, 1990 (Pages 309-314) 0891-7035/90$3.00+.00
Scanning Microscopy International, Chicago (AMF O'Hare), IL 60666 USA

SCANNING AND TRANSMISSION ELECTRON MICROSCOPY, AND ELECTRON PROBE ANALYSIS OF THE INTERFACE BETWEEN IMPLANTS AND HOST BONE

Osseo-coalescence versus Osseo-integration

G. Daculsi[1,*], R.Z. LeGeros[2], C. Deudon[3]

[1]Laboratoire de Recherche sur les Tissus Calcifies et les Biomateriaux
Faculte de Chirurgie Dentaire, Place Alexis Ricordeau, 44042 Nantes Cedex, France

[2]New York University College of Dentistry, 345 East 24th Street, New York, NY, 10010, USA

[3]Groupement D'Interet Scientifique GIS Biomateriaux, Nantes, France

(Received for publication November 5, 1989, and in revised form June 15, 1990)

Abstract

Bioinert materials (e.g., alumina implants) and bioactive ceramics (e.g., calcium phosphate ceramics, glass-ceramics) are now extensively used in dentistry. However, the physico-chemical interactions at the interfaces between the implant and the host bone are poorly understood. The purpose of this study was to define the interactions at these interfaces using a combination of analytical techniques: light microscopy, scanning and transmission electron microscopy, electron probe microanalysis, X-ray microradiography, X-ray diffraction, and infrared specstroscopy.

Bioinert (pure titanium) and bioactive materials (hydroxyapatite, beta-tricalcium phosphate and biphasic calcium phosphate) were implanted in dogs, and the implants, recovered after various periods of implantation, were analyzed.

The results demonstrated the following: the bioactive materials interact with the biological fluid and the living tissues in a specific manner. This process includes biodissolution/biodegradation, apatite crystal precipitation, and bone formation on the implant surface at the expense of the material. The results are discussed according to the limitations of the analytical techniques used.

The medical and chemical word COALESCENCE is suggested to describe the specific interactions of bioactive materials and INTERACTION for the phenomenon of physical contact of the bioinert materials with the host bone.

KEY WORDS: Calcium phosphate ceramics, bioactivity, interface, bone, osseo-coalescence, osseo-integration.

*Address for Correspondence:
Guy Daculsi,
Laboratoire de Recherche sur les Tissus Calcifies et les Biomateriaux, Faculte de Chirurgie Dentaire,
Place Alexis Ricordeau,
44042 Nantes Cedex,
France

Telephone: (33) 40 48 31 20
FAX: (33) 40 20 18 67

Introduction

The Conference of the European Society for Biomaterials (March 1986) on "Definitions in Biomaterials" was unable to define the word osseo-integration. There was no consensus among the participants on this term due to insufficient evidence for the development of an objective definition (34). The word osseo-integration was simultaneously used for the description of the interactions of both bioinert (alumina, titanium) or bioactive materials (calcium phosphate and glass ceramics) neglecting the important differences in the interface between the bone and the material (2, 7-9, 17, 18, 20).

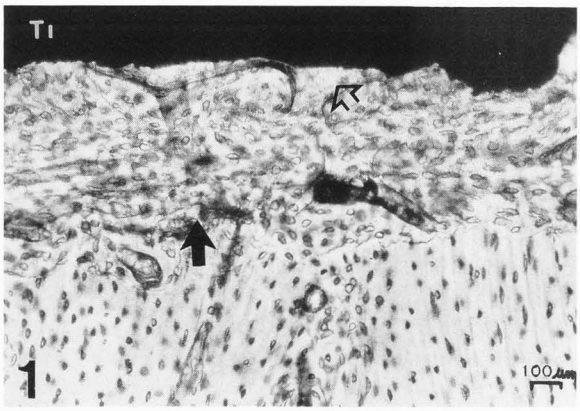
The purpose of this paper is to characterize the interactions between bioinert (e.g., titanium) and bioactive (e.g., calcium phosphate ceramics) and the host bone using physicochemical techniques (X-ray diffraction XRD, infrared (IR) spectroscopy, electron probe microanalysis), histological analyses (light, polarized microscopy, X-ray microradiography) and ultrastructural techniques (scanning (SEM) and transmission (TEM) electron microscopy, high resolution (HR) TEM) at the interface of the newly forming bone and implant surface.

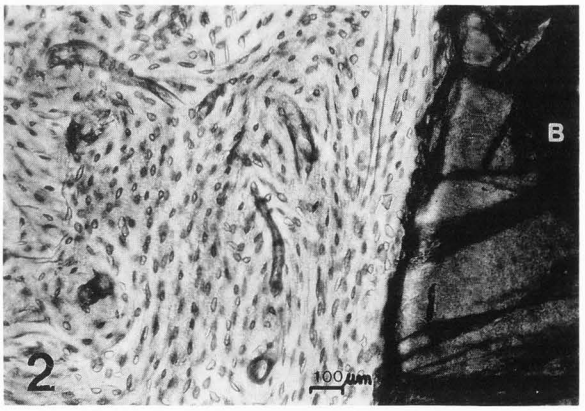
Materials and Methods

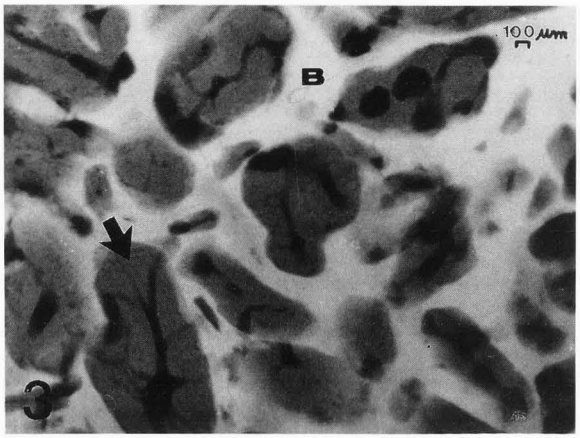
Pure titanium and well defined calcium phosphate ceramics (hydroxyapatite HA, beta-tricalcium phosphate b-TCP), and biphasic calcium phosphate ceramic (BCP) were prepared as 3x3 mm cylinders. Calcium phosphate ceramics were characterized by XRD and IR spectroscopy before and after implantation.

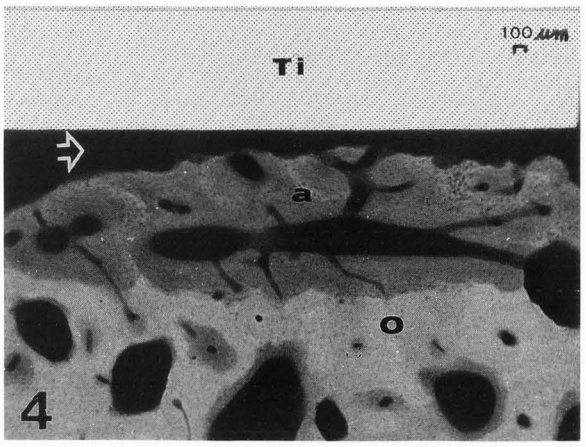
The titanium and the calcium phosphate ceramics were implanted in cortical bone of the femoral diaphysis of 7 dogs. The implants, recovered after three months, were characterized using histological methods, HR TEM, micro-electron diffraction, and electron probe microanalysis. For histological characterization, implant sections were embedded in methylmethacrylate, sectioned with a diamond saw and observed under polarized light microscopy. X-ray microradiography was performed at 15 kV. Then the sections were carbon coated by ion sputtering and examined by SEM at 15 kV. Electron probe X-ray microanalysis was performed at 10 kV.

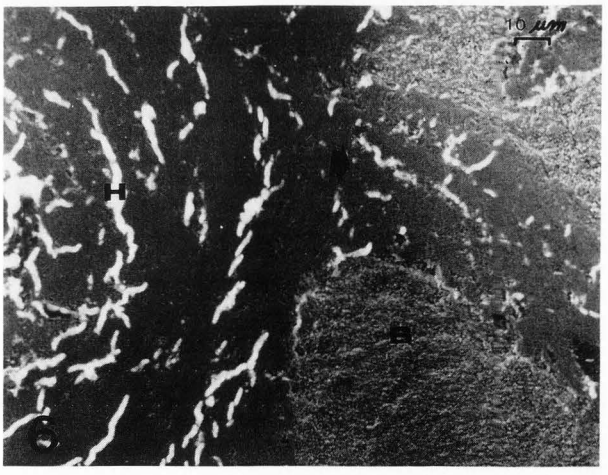
TEM analyses were performed on calcium phosphate ceramic implants only. Small undecalcified sections of the recovered implants were embedded in butyl-methylmethacrylate (1:1) and sectioned with a











Figures 3 and 4 (above middle). X-ray microradiographs of macroporous BCP (Fig. 3) and titanium (Ti, Fig. 4)

Figure 3. Bone formation (arrow) invading the macropores of the biomaterial (b) is observed.

Figure 4. A fibrous interposition (arrow) between the titanium surface and the newly forming bone (a) is observed. The old cortical bone (o) appeared more mineralized than the new bone.

Figures 5 and 6 (above bottom). SEM micrographs of ground sections of titanium (Ti, Fig. 5) and uncalcified BCP (Fig. 6) implant in femoral cortical bone.

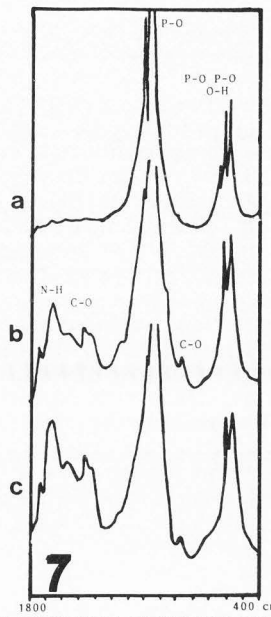
Figure 5. Displacement (arrow) of the Ti implant from the bony bed is observed.

Figure 6. Haversian bone (H) is observed in close contact with the biomaterial surface (B) and invading the macropore (arrow).

Figure 1 (facing page, top left). Pure Titanium implant in dog femoral cortical bone. New bone formation (dark arrow) is observed in contact with titanium, and in some areas of soft tissue imposition (light arrow).

Figure 2 (facing page, top right). Dense HA implant (B) in dog femoral cortical bone. New lamellar bone formation in direct contact with the ceramic surface is observed.

Figure 7 (at right). IR spectroscopy results of HA, BCP (HA/b-TCP 60/40) ceramics before (a), and after 3 months of implantation [HA (b), BCP (c)] showing the carbonate content (C-O).



diamond knife. HR TEM and micro-electron diffraction were performed at 200 kV with a TEMSCAN JEOL 200CX. Some undecalcified sections studied in HR TEM were decalcified by nitric acid, stained with uranyl acetate and lead citrate, and observed in TEM at 100 kV.

Results

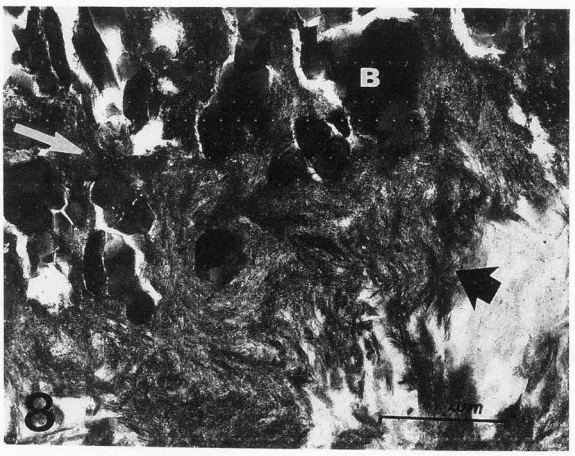
Standard histological analysis showed no significant differences between the apparent bone formation in contact with titanium (Fig. 1) and that in contact with calcium phosphate ceramics (Fig. 2).

Lamellar bone with regular osteocyte distribution was closely associated with the surface of the implants. X-ray microradiography demonstrated extensive mineralization of the new bone (Fig. 3). Titanium implants, or some limited surface areas of the titanium implants, were usually surrounded with soft tissues acting as a fibrous interposition (Fig. 4). Consequently, the titanium implants were easily displaced from the bony bed (Fig. 5) during the sectioning process. The electron microprobe revealed a high level of sulfur at the interface.

No fibrous encapsulation of the calcium phosphate ceramic implants was observed. The close association of bone and ceramic surfaces was preserved during the sectioning procedure. The bone growth penetrated into all free spaces and was observed directly on the ceramic surface (Fig. 6).

Microprobe analysis of the titanium implants before and after implantation indicated no changes in the material. With the calcium phosphate ceramics, however, an increase in the Ca/P ratio was observed after implantation (Table 1). Sulfur was not detected to be associated with calcium phosphate ceramic implants. The IR analysis of materials from the surface and the core of recovered implants showed carbonate-containing apatites (Fig. 7).

TEM observations of undecalcified sections of the contact zone between new bone and the surface of the calcium phosphate implant showed a mineralization of the dense collagen network (Fig. 8). New needle shaped crystals were closely associated with the three-dimensional distribution of the collagen fibers and, at the same time, were closely associated with the implant crystals. Electron micro-diffraction of the tiny crystals showed 0.27 nm rings corresponding to the 300 plane of apatite similar to the crystals of the host bone.



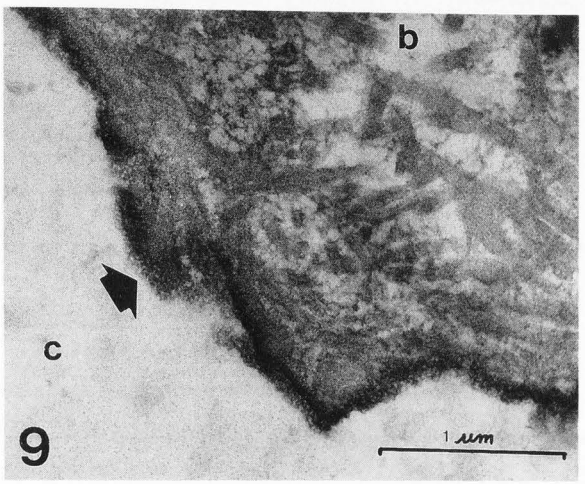


Figure 8. Undecalcified section of BCP implant observed in TEM showing the interface of the ceramic crystals (B) with the mineralized bone matrix (arrow).

Figure 9. Decalcified section of BCP (HA/b-TCP ratio 60/40) ceramic implant, stained with uranyl acetate and lead citrate, observed in TEM, showing an electron dense granular layer (arrow) at the interface of bone (B) and ceramic (C).

Table 1. Ca/P weight ratios (obtained from electron microprobe analyses) for HA, b-TCP, and BCP (HA/b-TCP ratio 60/40) ceramics before and after implantation in osseous site.

n = 10	HA	b-TCP	BCP
Before	2.15±0.05	1.94±0.05	2.06±0.08
After	2.36±0.09	2.32±0.10	2.37±0.10
***	10%	20%	15%

*** Differences highly significant less than 0.1.

Decalcified sections observed in TEM revealed an electron dense layer at the implant surface corresponding to the interface of bone and ceramic. This electron dense layer, showing a granular feature, was only observed on decalcified section stained with lead and uranyl salts (Fig. 9).

Another experiment (unpublished results) in Guinea pig using glass ceramic (Ceravital) for ossicular chain reconstruction was carried out with HR TEM. The results demonstrate some biodissolution and biological apatite crystal precipitation between the residual crystals of the materials. These newly formed crystals are similar to the biological crystals observed at the interface of bone and calcium phosphate ceramics.

Discussion

In vivo and in vitro biocompatibility has been demonstrated for titanium and titanium alloy (4, 29, 30) and for calcium phosphate ceramics (12, 21, 25, 32). A fundamental difference between these two materials is that the latter is resorbable (6-8', 10, 11, 22-24). The extent of dissolution depends on the hydroxyapatite (HA) or beta-tricalcium phosphate (b-TCP) content, HA is considered as the less resorbable (9, 12, 21, 22, 24).

Titanium has been successfully used for surgical implants in the jaw for a number of years. It has been shown that bone appears to grow in direct apposition to the titanium implant surface (1, 4, 29). The success of the dental implants is essentially due to a specific surgical technique introduced by Branemark under the label of OSSEOINTEGRATION: "Our principle for anchoring oral implants which we call osseo-integration, depends upon direct anchorage to the bone tissue and is entirely different from both subperiosteal and endosseous implantation, which predominantly depends on anchorage via non-mineralized connective tissue." (4). Starting from this definition, the definition of osseo-integration has been modified to refer to direct bone contact with a metallic implant.

Osseointegration has been defined by Wilson-Hench in "Definitions in Biomaterials" edited by Williams (34) as the process of combining new bone with a bioactive material. These materials, such as calcium phosphate or glass ceramic (17, 18), are able to induce specific biological activity. The calcium phosphate ceramics induce intra and extra cellular dissolution, bioresorption, early mineralization by biological apatite precipitation, and bone growth by osteoconduction on the implant surface (2, 5, 6, 10, 16, 19, 24, 27, 28). X-ray and IR characterizations of the calcium phosphate implant surface, both before and after implantation) have demonstrated the reactivity of the living tissue/calcium phosphate ceramics interface (9, 10, 19) incorporating biological ions (carbonate). These processes correspond to intimate chemical and biochemical interactions of the implant domains with the surrounding tissue, as in bone bonding (1, 9, 12, 14-18, 20, 21, 26, 27, 31), and not simply a mechanical interlocking. The medical and chemical term COALESCENCE is exemplified by the interactions produced by these bone formation processes, such as between biological apatite and calcium phosphate ceramic crystals.

Alternatively, bone bonding to titanium involves close contact and mechanical interlock between bone and titanium oxide (29, 30). This physical interaction corresponds to INTEGRATION. The electron microprobe indicated no change of the surface implant, however, in contrast to the bioactive materials, sulfur was detected at the interface, indicating sulfated organic compounds at the interface. The chemical aspect of calcium phosphate resorption, bone-like apatite crystal precipitation (expressing the bioactivity) and the osseo-conduction process seem specific to the bioactive ceramics (6, 9, 12, 21, 24, 26, 28).

The use of the word Osseo-integration to describe other materials implanted in osseous site (calcium phosphate ceramics in particular), is inappropriate as it neglects the bioactivity of these materials and the specific interface. Coalescence is the medical term to describe the bonding of tissues during wound healing, and chemical term for the accretion of discrete crystals into a new one. Coalescence seems to be more appropriate to describe the bone interaction with calcium phosphate and the specific interface of bioactive materials with the newly formed bone.

This study characterized the biomaterial/host bone interface using a combination of structural and ultrastructural analytical techniques. The use of several analytical techniques is necessary because of the limitations of each method. For example, artifacts may be introduced during any one of the following preparation processes: fixation, decalcification, embedding or sectioning. One example of artifact is the "electron dense layer" observed at the interface of bone and ceramic, described by Ganeles on decalcified sections (16). In our study, this apparent "electron dense layer" was only observed in TEM of decalcified sections when a staining agent was used. Using high resolution TEM, at the lattice pattern level, without any processing (fixation, decalcification and staining) this interface was never observed. Thus the "electron dense layer" must be regarded as an artifact: it may be representing debris of some proteins adsorbed on the implant surface like ghost crystals observed in dental enamel (3). Another serious limitation is the resolution of the technique used: 0.2 to 2 micrometers in light microscopy, 5 to 6 nm in SEM, 0.2 nm on crystals observed in HR TEM or 1 nm in organic material in TEM. It is not possible to observe single bone apatite crystals without a resolution similar to the crystal unit cell; or to observe a soft tissue interposition of 0.1 micrometer in light microscopy.

For physicochemical analysis, some limitations also need to be considered. IR or XRD materials scraped from the surface of the recovered implant include the interface, the transformed and original material. Electron microprobe is also limited by the electron beam diameter (1 micrometer for the area diameter in SEM, and 5 nm using TEMSCAN).

In conclusion, the interface between biomaterial implants and bone was difficult to analyze. It is necessary to use a combination of techniques from the macroscopy to atomic surface analysis, and to take into account the advantages and limitations of each method. Using a combination of techniques, this study demonstrates the interface characteristics between bioinert and bioactive implants with bone. We suggest that the physical term INTEGRATION (osseo-integration) be used to describe the phenomenon of mechanically functional contact defined by De Lange (13) and the biological and chemical term

COALESCENCE (osseo-coalescence) to describe the biological interactions of new bone and bioactive ceramics.

Acknowledgements

We wish to thank Mr. F. Guerin and Mr. J. Tolbert for their suggestions and contributions to this article; Prof. J.C. Le Nihouannen (National Veterinary School) and Prof. M. Basle (Medical Faculty of Angers) for their invaluable contribution.

References

1. Albrektsson T, Branemark PI, Hansson HA, Kasemo B, Larsson K, Lunstrom I, McQueen DH, Skalak R (1983). The interface zone of inorganic implants in vivo: titanium implants in bone. Ann. Biomed. Eng., 11, 1-27.

2 Amler MH (1987). Osteogenic potential of non vital tissues and synthetic implant materials. J. Periodontol. 58, 758-761.

3 Bonucci E (1985). Crystal matrix relationship in calcifying organic matrices. In: Morphogenese et differenciation dentaires, Belcourt AB, Ruch JV (eds.), INSERM, Paris, 125, 459-472.

4 Branemark PI, Hanson BO, Adell R, Breine U, Lindstrom J, Hallen O, Ohman A (1977). Osseointegrated implants in the treatment of the edentuleous jaw. Experiences from 10 years period. Scand. J. Plast. Reconstr. Sur. 16, 7-132.

5 Cameron HU, MacNab I, Piller RM (1977). Evaluation of a biodegradable ceramic implant. J. Biomed. Mater. Res. 11, 179-181.

6 Daculsi G, LeGeros RZ, Heughebaert M, Jans I (1988). Biological apatites associated with calcium phosphate ceramics implanted in osseous and non-osseous sites. J. Dent. Res. 67, 370 (abstract, full paper in reference 10 below).

7 Daculsi G, Orly I, Gregoire M, Heughebaert M, Hartmann DJ, Kerebel B (1986). Cell interactions with mixed calcium phosphate (TCP and HA) alumina solid phase: an ultrastructural study. In: Christel P, Meunier A, Lee AJC (eds.), Advances in Biomaterials Vol.IV, Biological and Biomechanical Performances of Biomaterials, Elsevier, Amsterdam, 337-342.

8 Daculsi G, Hartmann DJ, Heughebaert M, Hamel L, Le Nihouannen JC (1988). In vivo cell interactions with calcium phosphate. J. Submicroscop. Cytol. Path. 20, 374-384.

9 Daculsi G, LeGeros RZ, Nery E, Lynch K (1988). Transformation of biphasic calcium phosphate ceramics in vivo. J. Biomed. Mat. Res. 23, 883-894.

10 Daculsi G, LeGeros RZ, Heughebaert M, Barbieux I (1990). Formation of carbonate apatite crystals after implantation of calcium phosphate ceramics. Calcif. Tissue. Int. 46, 20-27

11 Daculsi G, LeGeros RZ, Mitre D (1989). Crystal dissolution of biological and ceramic apatites. Calcif. Tissue. Int. 45, 95-103.

12 De Groot K (1983). Bioceramics of calcium phosphate. CRC Press, Boca Raton, Florida.

13 De Lange G (1987). Discussion Session Interfacial Reactions. In: Definitions in Biomaterials, Williams DF (ed.), Elsevier, Amsterdam, 60-65

14 Ducheyne P, Van Raemdonck W, Heughebaert JC, Heughebaert M (1986). Structural analysis of hydroxyapatite coatings on titanium. Biomaterials, 7, 97-103.

15 Ducheyne P (1987). Bioceramics: Material characteristic versus in vivo behavior. J. Biomed. Mater. Res. 21, 219-236.

16 Ganeles J, Listgarten MA, Evian CI (1986). Ultrastructure of durapatite periodontal tissue interface in human intrabony defects. J. Periodontol., 57, 133-140.

17 Gross U, Schumacher D, Strunz V (1983). Comparative studies on the tissue reaction after implantation of glass ceramic into human, pig, rat, chicken bones. In: Ceramics in Surgery, Vincenzini P (ed.), Elsevier, Amsterdam, 161-168.

18 Hench LL, Splinter RJ, Allen WC, Greenlee TK (1971). Bonding mechanisms at the interface of ceramic prosthetic materials. J. Biomed. Mater. Res., 2, 117-141.

19 Heughebaert M, LeGeros RZ, Gineste M, Guilhem A (1988). Physico-chemical characterization of deposits associated with HA ceramics implanted in non-osseous sites. J. Biomed. Mater. Res., 22, 257-258.

20 Jarcho M, Kay JF, Drobeck HP, Doremus RH (1976). Tissue, cellular, and subcellular events at bone-ceramic hydroxylapatite interface. J. Bioeng., 1, 79-92.

21 Jarcho M (1981). Calcium phosphate ceramics as hard tissue prosthetics. Clin. Orthop. 157, 259-278.

22 Klein CPAT, Driessen AA, De Groot K, Van Den Hoof A (1983). Biodegradation behavior of various calcium phosphate materials in bone tissue. J. Biomed. Mater. Res., 17, 769-784.

23 Klein CPAT, Driessen AA, De Groot K (1984). Relationship between the degradation behavior of calcium phosphate ceramics and their physical chemical characteristics and ultrastructural geometry. Biomaterials. 5, 157-160.

24 LeGeros RZ, Parsons R, Daculsi G, Driessens F, Lee D, Metsger S (1988). Biodegradation/bioresorption of calcium phosphate ceramics: task group report. In: Bioceramics: Material Characterization vs. In Vivo Behavior, Ducheyne P, Lemons J (eds.). New York Acad. Sci., 253, 268-271.

25 LeGeros RZ (1988). Calcium phosphate materials in restorative dentistry: a review. Adv. Dent. Res., 2, 164-180.

26 Nery EB, Lynch KL (1978). Preliminary clinical studies of bioceramic in periodontal osseous defects. J. Periodont., 49, 523-527.

27 Osborn JF, Newesely H (1980). The material science of calcium phosphate ceramic. Biomaterials, 1, 108-111.

28 Passuti N, Daculsi G, Rogez JM, Martin S, Bainvel JV (1989). Macroporous calcium phosphate ceramic performance in human spine arthrodesis in humans and dogs. Clin. Orthop. Rel. Res. 248, 169-176.

29 Sundgren JE, Bodo P, Lunstrom I (1986). Auger electron spectroscopic studies of the interface between human tissue and implants of titanium and stainless steel. J. Coll. Interf. Sci., 110, 9-20.

30 Toth RW, Parr GR, Gardner LK (1985). Soft tissue response to endosseous titanium oral implants. J. Prosthet. Dent., 54, 564-567.

31 Tracy BM, Doremus RH (1984). Direct electron microscopy studies of the bone hydroxylapatite interface. J. Biomed. Mater. Res., 18, 719-726.

32 Van Raemdonck W, Ducheyne P, De Meester P (1984). Calcium phosphate ceramics. In:

Metals and Ceramic Biomaterials, Ducheyne P, Hastings G (eds.), CRC Press, Boca Raton, Florida, 144-166.

33 Warshawsky H, Bai P, Nanci A, Josephsen K (1984). Morphological visualization of two categories of enamel proteins in relation to the crystals of rat incisor enamel. In: Fearnhead RW, Suga S (eds.), Tooth Enamel IV, Elsevier, Amsterdam, 177-182.

34 Williams DF (1987) ed. Definitions in Biomaterials, Elsevier, Amsterdam, 1-72.

Discussion with Reviewers

U. Gross: The abstract mentions biodissolution/biodegradation of bioactive materials. What is the evidence for these processes?
Authors: We have demonstrated in previous papers (8-11, 28) the dissolution/degradation process in bioactive ceramics using TEM (extra-cellular and intra-cellular dissolution by phagocytosis). The present paper refers to these works. Other studies on Ca-P ceramic dissolution have also been reported by several authors (22, 23, 34).

U. Gross: How do the authors explain calcium phosphate resorption and apatite crystal precipitation at the interface?
Authors: The formation of bone apatite-like crystals is due to the precipitation of calcium and phosphate ions released from the dissolving ceramic crystals at the surface of residual crystals (epitaxial growing process) and into the micropores by secondary nucleation. The phenomena depends on the Ca and P saturation of the fluid penetrating the intercrystalline spaces (micropores). It is essentially a physicochemical process alternating from saturation/precipitation to a new equlibrium back to saturation/precipitation.

We have observed precipitation in some part of the implant and dissolution in other parts, corroborating the alternative dissolution/precipitation process.

U. Gross: Does the precipitated material originate from the implant or from the intersticial fluid?
Authors: We cannot indicate the exact origin of the ions precipitating into carbonated apatite crystals; however, it is evident that CO_3 and Mg originate from biological fluid. A double origin (implant and biological fluid) must be considered for Ca and P (6, 9, 10), the precipitation occurs in the biological fluid by secondary nucleation, and at the crystal surface of the implants by epitaxic growing process.

E. Bonucci: Although osteoclastic resorption is often present in bone facing the implant, you do not mention this process at all. Was it completely absent?
Authors: It is true that osteoclastic resorption are observed facing the implant. Generally this is observed during the bone remodelling after 1.5 to 3 months of calcium phosphate implantation.

E. Bonucci: The mechanical forces can greatly influence the interaction between implanted material and host tissue and consequently can change the reaction of the latter to the presence of the former. You report that bioinert and bioactive materials were implanted in cortical bone, without specifying in which skeletal segment and in which site of that segment they were implanted. However, it is possible that the results vary according to the site of implantation and the mechanical forces exerted on it. Can you comment on this possibility?
Authors: It is evident that the mechanical forces modify the osseo-coalescence and osseo-integration process. For this reason, we have selected the diaphyseal part of the femoral cortical bone, less submitted to mechanical forces than the epiphyseal part.

In this case, we have probably compared the bioactive properties of the materials.