JOURNAL OF MATERIALS SCIENCE: MATERIALS IN MEDICINE **14** (2003) 195–200

Current state of the art of biphasic calcium phosphate bioceramics

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We have developed 15 years ago, with the collaboration of Lynch, Nery, and LeGeros in the USA, a bioactive concept based on biphasic calcium phosphate (BCP) ceramics. The concept is determined by an optimum balance of the more stable phase of HA and more soluble TCP. The material is soluble and gradually dissolves in the body, seeding new bone formation as it releases calcium and phosphate ions into the biological medium.

The bioactive concept based on the dissolution/transformation processes of HA and TCP has been applied to both Bulk, Coating and Injectable Biomaterials. The events at the calcium phosphate (CaP) biomaterial/bone interface represent a dynamic process, including physico-chemical processes, crystal/proteins interactions, cells and tissue colonization, bone remodeling, finally contributing to the unique strength of such interfaces. An important literature and numerous techniques have been used for the evaluation of the fundamental physico chemical and biological performance of BCP concept. This type of artificial bone used from a long time in preclinical and in clinical trial, revealed the efficiency for bone filling, performance for bone reconstruction and efficacy for bone ingrowth at the expense of the micro macroporous BCP bioceramics.

The development of calcium phosphate ceramics and other related biomaterials for bone graft involved a better control of the process of biomaterials resorption and bone substitution. Synthetic bone graft materials are available as alternatives to autogeneous bone for repair, substitution or augmentation. Synthetic biomaterials include essentially special glass ceramics described as bioactive glasses; calcium phosphates (calcium hydroxyapatite, HA; tricalcium phosphate, TCP; and biphasic calcium phosphate (BCP)). These materials differ in composition and physical properties from each other and from bone [1–4]; and must be take into consideration for more efficient bone ingrowth at the expense of the biomaterials and to adapt to new development of dedicated biomaterials.

We have developed 15 years ago, with the collaboration of Lynch, Nery, and LeGeros in USA, a bioactive concept based on BCP ceramics. The concept is determined by an optimum balance of the more stable phase of HA and more soluble TCP. The material is soluble and gradually dissolves in the body, seeding new bone formation as it releases calcium and phosphate ions into the biological medium [5–8]. BCP bioceramics consists of a mixture of hydroxyapatite (HA), $Ca_{10}(PO_4)_6(OH)_2$ and beta-tricalcium phosphate (β-TCP), $Ca_3(PO_4)_2$ of varying HA/β-TCP ratio. LeGeros initiated in USA basic studies on preparation of BCP and their *in vitro* properties in 1986 and Daculsi in France. At the present time, BCP is commercially available in Europe, Brazil, Japan, USA, Australia as a bone-graft or bone substitute materials for orthopaedic and dental applications under various trade mark (BCP®, MBCP®, Triosite®, Hatric®, Eurocer®, Biceram®, Bicalfoss® ...). It is now available in blocks, particulates, customized design (Fig. 1) and as an injectable material in a polymer carrier (Fig. 2).

BCP is obtained when a synthetic or biological calcium deficient apatite (CDA) is sintered at temperatures above 700 °C. The extent of calcium deficiency (Ca/P molar ratio < 1.67) depends on the method of preparation (by precipitation, hydrolysis or mechanical mixture), the reaction pH and temperature in the preparation of the unsintered apatite. The calcium deficiency determines the HA/β-TCP ratio in the BCP. The HA/β-TCP ratio in the BCP determines its reactivity [6, 8–10]: the lower the ratio, the higher the reactivity (expressed *in vitro* as the extent of dissolution in an acid buffer). Particle size, macro porosity and micro porosity (Figs. 3(a) and (b)) are also factors in the reactivity of BCP. Sintering temperature and conditions affect these properties.

The interest of BCP concept is the controlled dissolution and due to the structure, the bone ingrowth at the expense of the ceramic. Between 1920 and 1975, a very limited number of scientific articles reported that the use of calcium phosphate materials, described as ''tricalcium phosphate'', to repair bone defects successfully promoted bone formation [11, 12]; or periodontal

Figure 1 MBCP® block, granules, cylinders, wedges and customized design available for bone reconstruction.

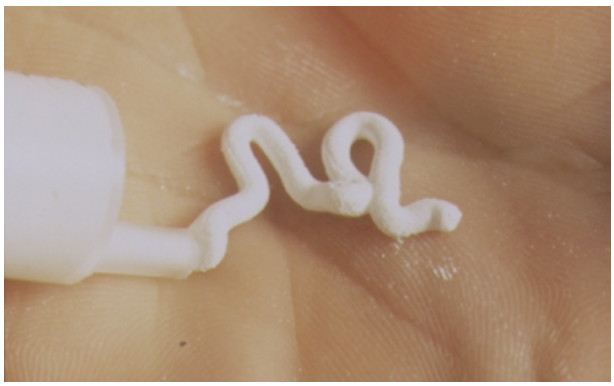

Figure 2 Injectable Bone Substitute IBS™, MBCPgel® composite of BCP granules and hydrosoluble HPMC polymer.

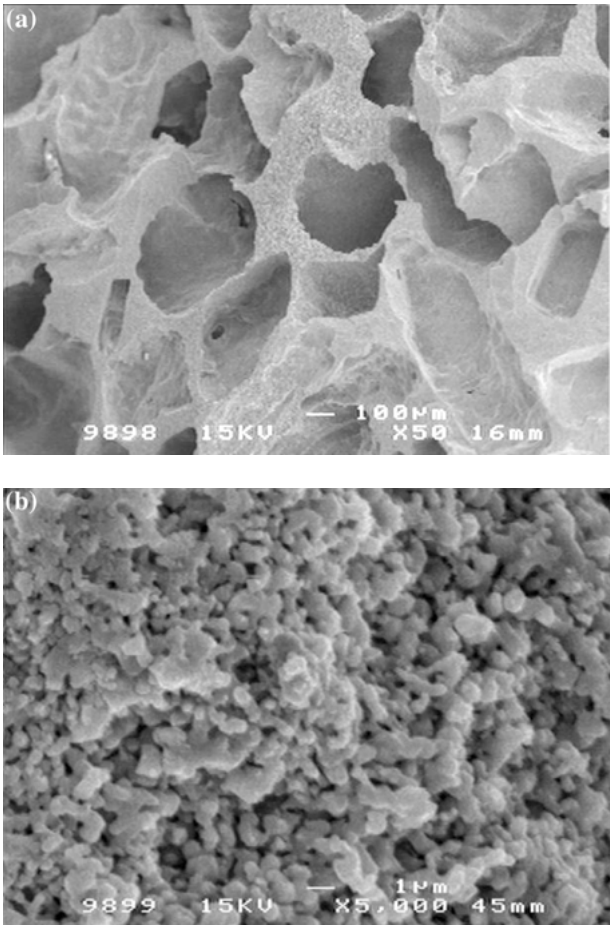

Figure 3 SEM of Triosite® blocs showing macroporous structure (a) and micropore (b).

defects [13]. The ''tricalcium phosphate'' material used by Nery was subsequently identified by LeGeros in 1988 as consisting of a mixture of 20% β-TCP and 80% HA [18]. This material and other mixtures of ß-TCP and HA were later described as a BCP.

The main attractive feature of bioactive bone graft materials such as BCP ceramic is their ability to form a strong direct bond with the host bone resulting in a strong interface compared to bio inert or bio tolerant materials which form a fibrous interface [1, 2, 14, 15].

The formation of this dynamic interface is believed to result from a sequence of events involving interaction with cells; formation of carbonate hydroxyapatite CHA (similar to bone mineral) by dissolution/precipitation processes.

Cellular events

The BCP materials elicit responses from bone cells and related cells *in vitro* and *in vivo* that are similar to those elicited by bone. These materials allow cell attachment, proliferation and expression. The first biological events after BCP ceramics implantation are biological fluid diffusion, followed by cells colonization. These cells are macrophages, in early steps, followed by mesenchymal stem cells, osteoblasts, osteoclasts, into the macropores of the implants (Fig. 4). The resorbing cells forming both at the surface of the newly formed bone and the bioceramic surface looks like osteoclast and are TRAP positive (Fig. 5). In human spine arthrodesis we have demonstrated what after a couple of months bone remodeling occurs, with secondary osteoclast resorption of the artificial bone and bone ingrowth at the expense of the implant (Fig. 6).

Generally when granules are used in osteo-articular surgery, some grains will be released in cartilage or non osseous site. Neither it was described foreign body reaction and rejected materials. Resorption or tissue incorporation was demonstrated. For example, in human spine arthrodesis after 3.5 months of implantation, granules of Triosite® appears surrounded by newly

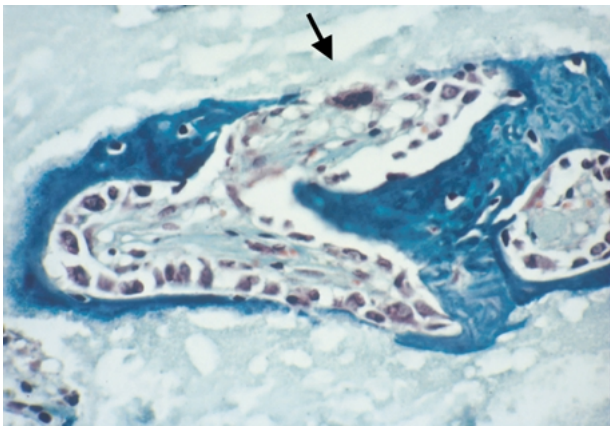

Figure 4 Newly formed bone into MBCP™ or Triosite® macropore in femoral epiphysis of rabbit after 14 days of implantation showing osteoclasts (arrow) and osteoblasts. Decalcified section stained with Masson's Trichromic staining.

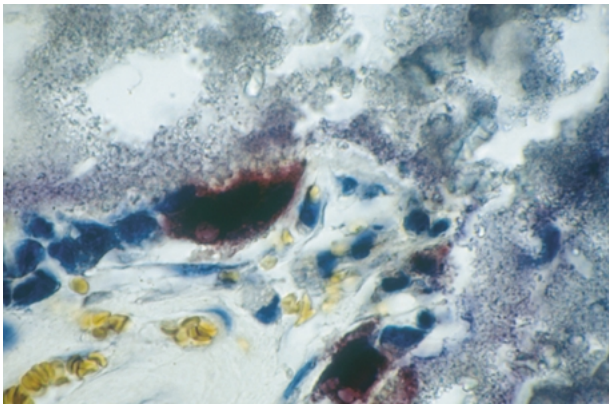

Figure 5 TRAP staining of osteoclast in femoral epiphysis of rabbit after 14 days of implantation.

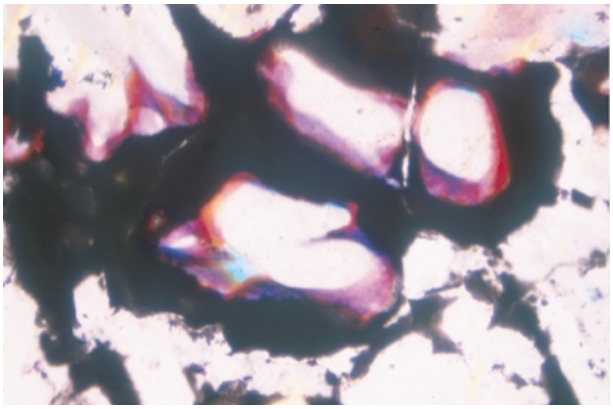

Figure 8 MBCP® granules implanted 2 weeks in muscular area of rabbit. Non-decalcified section with Movat's staining.

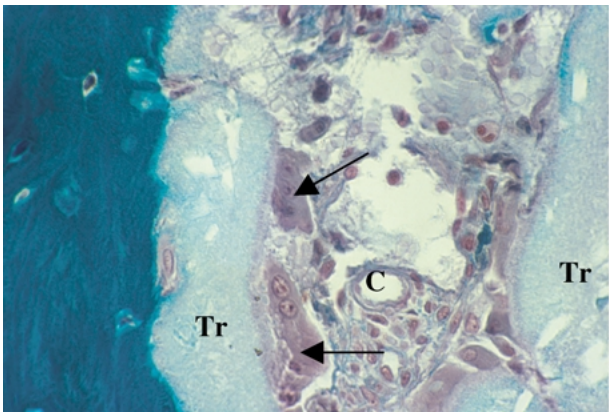

Figure 6 Human spine arthrodesis using Triosite® blocks after 3.5 months of implantation showing bone ingrowth at the expense of the Triosite® (Tr) with osteoclast (arrow) near vascular channel (C).

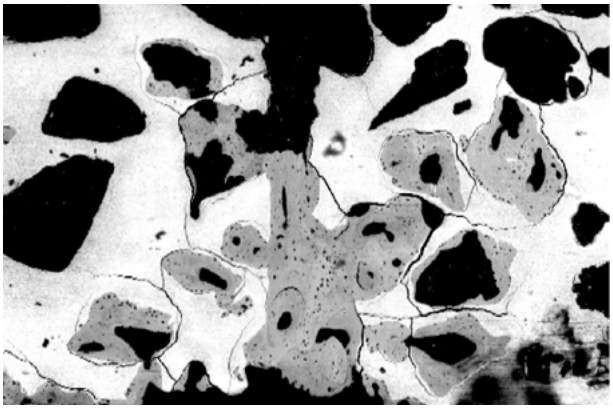

Figure 9 Bone reconstruction into MBCP® implant associated with autologous bone marrow and implanted in 65 grays irradiated femoral canine bone defects.

formed cartilage without fibrous encapsulation (Fig. 7). Moreover, in non osseous site after implantation in subcutaneous area, we have sometimes observed into some macropores of micro macroporous biphasic calcium phosphate (MBCP®) osteoid formation (Fig. 8). These observations suggest that BCP with macropores present suitable chemical environment associated to efficient architecture able to catch mesenchymal stem cells and to induce their phenotype to osteogenic cell lines. These observations have been also described by other groups in Netherlands [16]. This property can be used for artificial bone in irradiated implantation site. Irradiation produces irreversible effects on normal tissues, involving damages on their reparation properties. Nevertheless quality of life of patients who undergo radiotherapy could be improved by bone reconstructions. A preclinical study performed in irradiated dogs demonstrated bone ingrowth at the expense of structured implants of micro macroporous biphasic calcium phosphate filled by autologous bone marrow after implantation in irradiated soft and bone tissue [17] (Fig. 9).

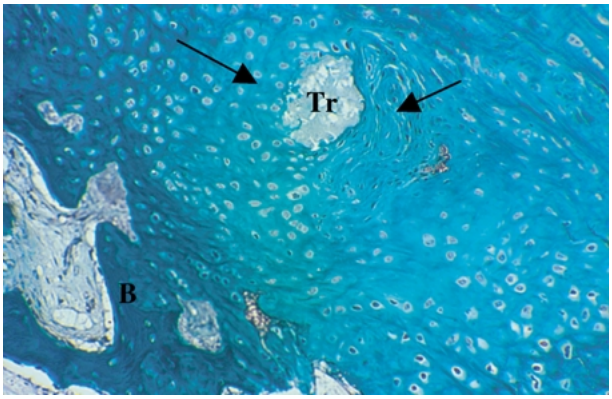

Figure 7 Human spine arthrodesis after 3.5 months of implantation showing hyaline and fibrous cartilage (arrows) growth all around a granule of Triosite® (Tr) and close to the newly formed bone at the expense of the implant (B).

Biodegradation, biodissolution and biological apatite precipitation significance

The biodegradation of BCP included the dissolution of the individual HA or β-TCP crystals [6, 9, 10]. The proportion of HA to β-TCP crystals in BCP appeared greater after implantation [18] and the known higher reactivity or solubility of β-TCP compared to HA.

The resorbability (reflecting *in vivo* dissolution) of BCP ceramics depends on their β-TCP/HA ratios, the higher the ratio, the greater the resorbability [6, 19]. Formation of microcrystals (which are able to diffract X-rays) with Ca/P ratios similar to those of bone apatite crystals was also observed after implantation. The abundance of these crystals was directly related to the

initial β-TCP/HA ratio in the BCP: the higher the ratio the greater the abundance of the microcrystals associated with the BCP crystals. According to these data it is possible to control the kinetic of dissolution and precipitation, and subsequently the bioactivity [20].

Using high resolution TEM Daculsi *et al*. [7] demonstrated for the first time that the formation of these microcrystals after implantation were non-specific, i.e., not related to implantation site, subjects of implantation, and types of CaP ceramics (Fig. 10).

The coalescing interfacial zone of biological apatite and residual crystals provides a scaffold for bone-cell adhesion and further bone ingrowth [18]. The resorbing process involves dissolution of calcium phosphate crystals and then a precipitation of CHA needle-like crystallites in micropores close to the dissolving crystals. The coalescing zone constitutes the new biomaterial/bone interface, which includes the participation of proteins and CHA crystals originating from the CaP materials, but does not include the biomaterial surface. The following events of bone ingrowth and the newly formed bone progressively replaces the initially formed CHA from the CaP biomaterials.

The process of cell colonization, adhesion, phagocytosis and osteoclastic resorption, Extra Cellular Matrix (ECM) elaboration and mineralization, bone in growth and bone remodeling associated with the biological apatite precipitation during CaP ceramics dissolution, are continuously in progress. Consequently the interface is not static but dynamic, in constant evolution, taking into account bone physiopathology, biomechanical factors and bone maturation. The processes involve a well organized and mineralized bone ingrowth at the expense of the artificial bone (Fig. 11). X-rays microtomography (micro scanner imaging) of the bone ingrowth at the expense macroporous BCP is able to demonstrate the three-dimensional bone organization into the macropores implant (Figs. 12(a) and (b)).

This concept of bioactivity could also be applied to implant coating and to Injectable Bone Substitute MBCPgel® [20]. CaP materials are also used as components or fillers in polymeric composites [21, 22] and in cements [23]. The hydraulic cement are not macroporous and numerous studies have demonstrated the necessity of macropores for bone osseous-conduction [4]. The bioactive concept of BCP have been applied to a new composite associating hydrosoluble polymer and BCP granules [24]. We have elaborated such injectable bone substitute ready to use and able to be largely invaded by osseous-conduction due to osteogenic cells [15]. These materials are perfectly biocompatible and potentially resorbable and, thanks to their initial plasticity, they can fit bone defects very easily, without

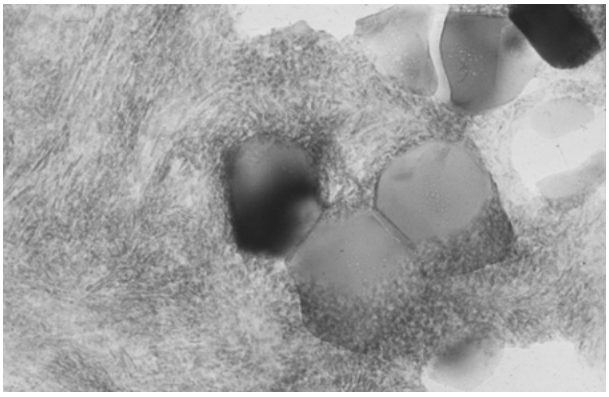

Figure 10 Biological apatite precipitation at the surface of residual crystals in BCP observed in high resolution TEM.

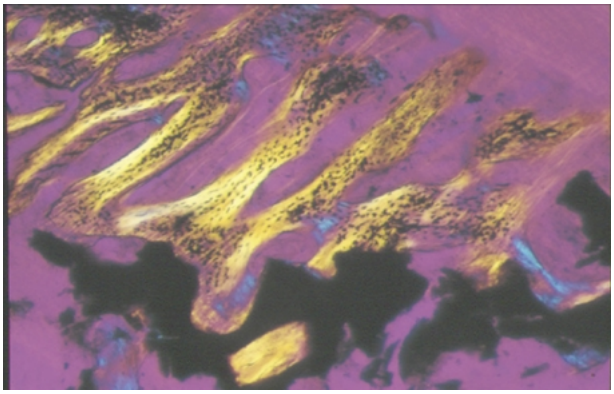

Figure 11 Spongious bone formed at the expense of Triosite® blocks in human spine arthrodesis.

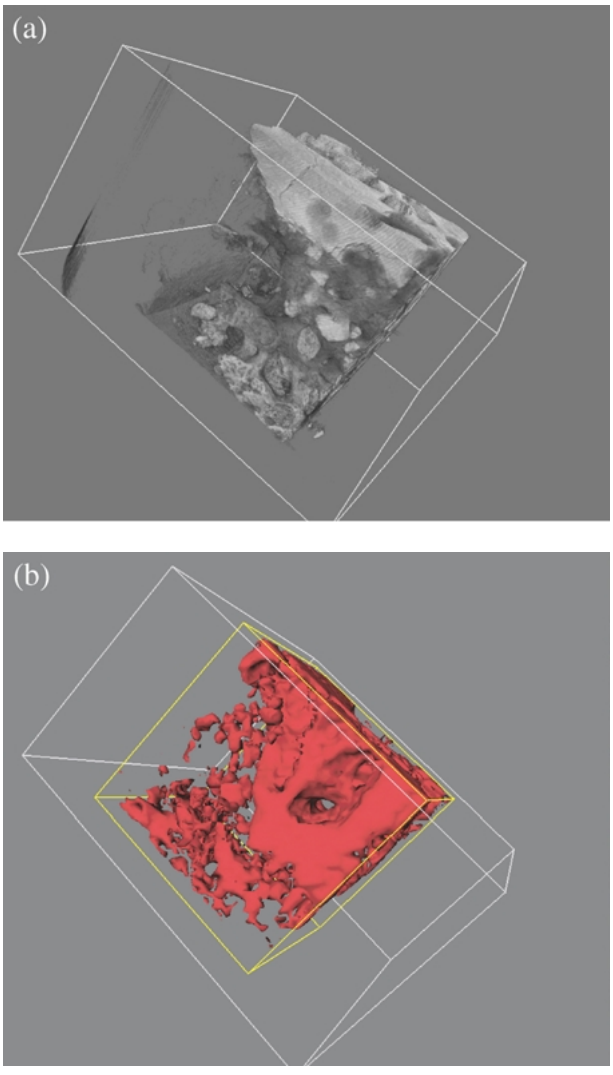

Figure 12 X-ray microtomography (Synchrotron facility, ESRF Grenoble France) of macroporous BCP implant in rabbit femoral epiphysis. (a) Total imaging with bone ingrowth (gray level) at the expense of bioceramics (white). (b) 3-D image reconstruction of bone ingrowth alone.

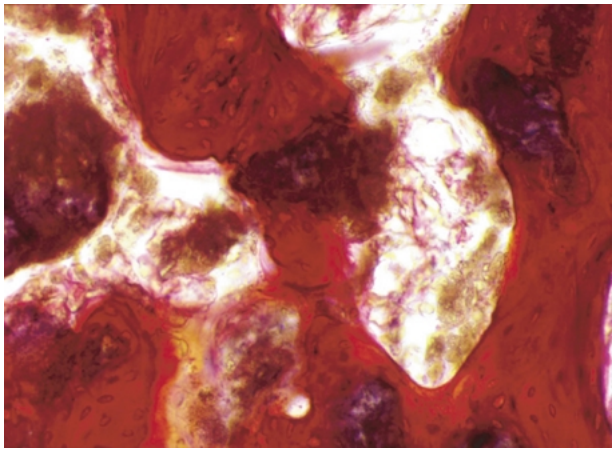

Figure 13 Cancellous bone formed into rabbit femoral epiphysis after 3 weeks of implantation of IBS® (Movat's staining).

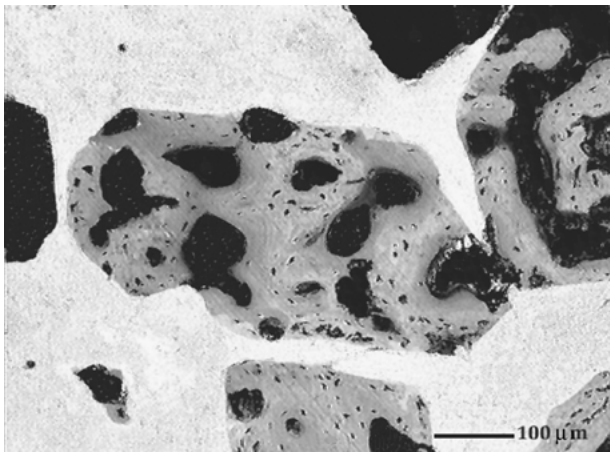

Figure 14 Scanning electron microscopy using Backscattered Electron imaging of polished section of MBCPgel® after 3 week of rabbit implantation showing bone growth closely associated to the residual grain of calcium phosphate.

necessity to elaborate shaping of implantation site [25, 26].

The IBS cannot have mechanical properties like hydraulic bone cement able to have a hardening process [23]. However bone cells are able to invade the spaces released by the disappearance of the polymer. Bone ingrowth take place all around and at the expense of the resorption of the BCP grains (Figs. 13 and 14). In time, mechanical property could be observed due to the presence of bone.

Conclusion

The bioactive concept based on the dissolution/transformation processes of HA and TCP can be applied to both Bulk, Coating and Injectable Biomaterials. The Biphasic Calcium Phosphate concept based on the mixture of HA and β-TCP in the three different forms have the same evolution and adaptation to the tissues: (1) partial dissolution of the CaP ceramic macrocrystals cause an increase in the calcium and phosphate concentrations in the local microenvironment; (2) formation of CHA (either by direct precipitation or by transformation from one CaP phase on an other or by seeded growth) incorporating ions (principally carbonate) from the biological fluid during its formation; (3) association of the carbonate-apatite crystals with an organic matrix; and (4) incorporation of these microcrystals with the collageneous matrix in the newly formed bone (in osseous sites). The events at the CaP biomaterial/bone interface represent a dynamic process, including physico-chemical processes, crystal/proteins interactions, cells and tissue colonization, bone remodeling, finally contributing to the unique strength of such interfaces. These type of artificial bone revealed from a long time in preclinical and in clinical trial the efficiency for bone filling, performance for bone reconstruction and efficacy for bone ingrowth at the expense of the micro macroporous biphasic calcium phosphate bioceramics.

Acknowledgment

The individual and collaborative studies were supported by research grants from the INSERM U225, CJF 93-05 and E 99-03 and CNRS EP 59 (Dr G Daculsi, Director) and from the National Institute for Dental Research of the National Institutes of Health Nos. DE04123 and DE07223 and special Calcium Phosphate Research Funds (Dr RZ LeGeros, Principal Investigator). The X-ray microtomography and 3-D imaging have been performed with the ESFR Grenoble France facilities.

We thank BIOMATLANTE (Vigmeure ok Bulagne France) and ZIMMER France for samples providing.

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Received 31 July
and accepted 31 October 2002