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# Biphasic Calcium Phosphate/Hydrosoluble Polymer Composites: A New Concept for Bone and Dental Substitution Biomaterials

G. DACULSI,[1] P. WEISS,[1] J.-M. BOULER,[1] O. GAUTHIER,[1,2] F. MILLOT, and E. AGUADO[1,2]

[1]*Centre de Recherche sur les Matériaux d'Intérêt Biologique INSERM, E99-03, Faculté de Chirurgie Dentaire, Nantes, France*
[2]*Service de Chirurgie, Ecole Nationale Vétérinaire de Nantes, Nantes, France*

**Calcium phosphate materials have been increasingly employed in orthopedic and dental applications in recent years and are now being developed for use in noninvasive surgery or as carriers for drug delivery systems. We developed an injectable bone substitute (IBS) constituted of biphasic calcium phosphate and a hydrosoluble polymer as a carrier. In vivo biocompatibility and biofunctionality of IBS were tested in rabbits using implants in osseous and nonosseous areas. The results obtained demonstrated that the concept of IBS, a filler without initial mechanical properties but able to be rapidly resorbed and replaced by newly formed bone, can be applied to new surgical applications in orthopedic surgery, maxillofacial surgery, and dentistry for pulp capping and root filling. (Bone 25:59S–61S; 1999)** 

**Key Words:** Injectable bone substitute; Biphasic calcium phosphate; Biomaterials.

## Introduction

In recent years, self-setting calcium phosphate cements have been of considerable interest in orthopedic and dental applications.[3,4,12,13,14,18] Calcium phosphate materials have also been used as components or fillers in polymeric composite,[2] in association with polymers such as polysaccharide.[1] However, these products have not proved efficient for bone osteoconduction and ingrowth.[17] Yet, it has been clearly demonstrated that macropores are required for bone ingrowth.[6,8,10]

The aim of this study was to present general data obtained from 7 years of research development on a new injectable bone substitute (IBS)[11,15] that could be efficiently colonized by osteogenic cells and rapidly replaced by newly formed bone.

## Materials and Methods

Methylhydroxypropylcellulose with an average molecular weight of 700,000 Da, was prepared in a 2% solution (w/w) by dissolving the powder in bidistilled water. Biphasic calcium phosphate (BCP) granules (60% hydroxyapatite [HA], 40% β-tricalcium phosphate [β-TCP]) with a grain size of 80 to 200 μm were selected. The w/w ratio was 60% for the mineral phase and 40% for the polymer solution. The BCP powder was sterilized by steam at 120°C for 1 h. The polymer solution was prepared in a clean room (class 100) in sterile conditions.

Eighty adult New Zealand white rabbits, 28 rats Sprague Dawley, 15 guinea pigs, 11 beagle dogs, and 28 sheeps were used for osseous and nonosseous implantation sites. The animals were killed from 1 to 78 weeks after implantation. The recovered samples were fixed with 10% neutral formaldehyde solution. Some samples were decalcified for histological studies, and stained using the Masson-Goldner trichromic method. The other samples were embedded in methylmethacrylate and sectioned using a diamond saw into 50- to 100-μm-thick slices. The slices were stained using Movat's pentachrome and observed in normal and polarized light microscopy. X-Ray microradiography was performed on these same sections. After diamond saw sectioning, residual blocks were examined by scanning electron microscope (SEM) operating at 15 kV, and using back-scattered electron imaging (BSE). Image analysis was performed on sections using a Quantimet 500 system (Leica, Cambridge, UK).

## Results

The optimal ratio for the BCP/polymer solution was 60/40 (w/w). The microporosity, as determined by image analysis from SEM observations, represented 33% ± 0.5% of the grain surface area. The implantation in nonosseous sites (subcutaneous, intramuscular site) from a short-time implantation period (1 week) to 78 weeks indicated no inflammatory reaction or fibrosis. A homogeneous degradation front was observed between the BCP grains, particularly for the smallest particles from the surface to the core (**Figure 1**). The nondegradated or resorbed granules on the surface are mainly over 80 μm in diameter. This result is regularly observed in all samples, both in osseous and nonosseous implantation sites.

In osseous implantation sites, after 1 and 2 weeks of implantation, a decrease in the density of the biomaterial surface was observed in conjunction with the organization of woven bone matrix all around the larger particles. With time, bridges of woven bone appeared between the granules, and trabecular bone was observed all around and between the larger particles (>80 μm) (**Figure 2**). These residual granules appeared to be completely covered with lamellar bone (**Figure 3**). Spaces previously occupied by the polymer were totally filled by newly formed bone, and smaller particles were no longer observed.

In rabbits after 12 weeks of implantation time, the total surface area occupied by newly formed bone and residual BCP

*Address for correspondence and reprints:* G. Daculsi, Faculté de Chirurgie Dentaire, Place A. Ricordeau, 44042 Nantes Cedex, France.





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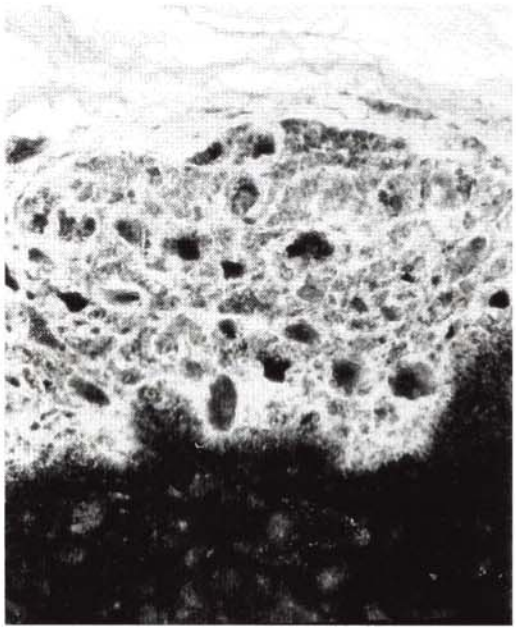

**Figure 1.** Nondecalcified section of IBS implanted in nonosseous site in rabbit (subcutaneous) after 4 weeks. No fibrous encapsulation was observed in foreign body reactions. The degradation of the material occurs from the periphery to the core of the filled defect. The residual granules are mainly over 80 μm in diameter (original magnification ×30).

grains was 79%. The size of these residual grains was 104 ± 32 μm in diameter. The same kinetics of resorption and bone substitution was observed in other animal experiments.

## Discussion

Injectable bone substitute cannot be compared with ionic bone cement, which is able to undergo a hardening process after mixture of the components.[4,12,14,16,17] However, bone cells in injectable bone substitute (IBS) are able to invade the spaces created by the disappearance of the polymer, so that general bone ingrowth occurs to the detriment of BCP grain resorption. The disappearance of the polymer will be due to diffusion, and solubility in extracellular body fluid, or/and cellular activity. In time, a mechanical property can be observed due to the presence of bone.[19] Cellulose and its derivatives have long been used for medical purposes and can be selected to achieve this goal.

Implantation in trabecular bone in our study was justified to test the efficiency of IBS in filling bone defects without large diffusion into trabecular bone, and to determine the resorption and bone replacement that occur to the detriment of such material. To answer certain questions raised by surgeons concerning the behavior of BCP granules during injection in nonbony sites, we also tested biocompatibility in subcutaneous and intramuscular implantation sites. The results demonstrate no foreign body reaction and no ectopic bone formation.

The hydrosoluble polymer was mixed with calcium phosphate, a material well known for its osteoconduction properties[8] and ability to provide a scaffold for cell spreading. The efficiency of BCP is related to its capacity for partial dissolution, the long-term stability of HA, and the enhanced bioactivity of readily soluble TCP.[5,7,10] Methylhydroxypropyl cellulose was chosen for its pseudoplastic properties and is considered to have only a carrier effect and a spacer to preserve spaces between the granules for angiogenesis and osteoconduction.

Direct contact between the biomaterials and soft tissues was achieved without encapsulation or fibrosis. The biofunctionality of IBS relates to its two levels of porosity. The microporosity of BCP grains is necessary for fluid circulation and to induce the dissolution/precipitation process for calcium phosphate crystals.[9] In our study, residual particles were about 100 μm in diameter 3 months later, which would appear to be a minimal or critical size to be a scaffold for cell adhesion. The mechanism of hydrosoluble polymer (HPMC) disappearance is still unknown.

It may be concluded that injectable HPMC-BCP ceramic constitutes a truly new type of bone filler, which could provide an alternative to ionic cement for obtaining bone ingrowth within the core of the material. Moreover, this ready-to-use IBS, like macroporous ceramic, can be colonized by osteogenic cells.

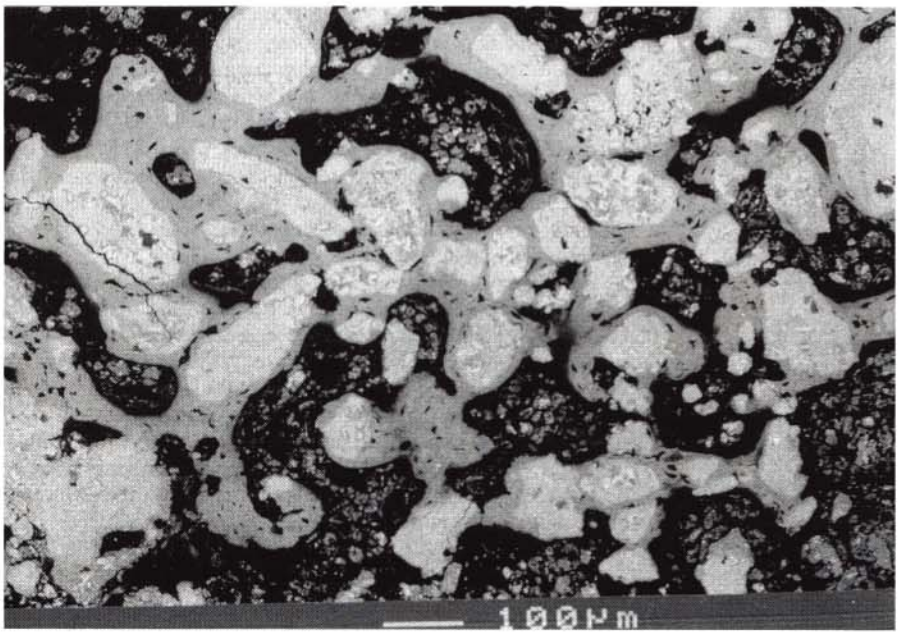


**Figure 2.** SEM examination using BSE of IBS after 4 weeks of implantation in distal part of rabbit femur shows the bone ingrowth at the expense of the composite, surrounding the nondegraded larger particle. The bone ingrowth progresses by osteoconduction at the surface of the granules, which act as a scaffold (original magnification ×100).

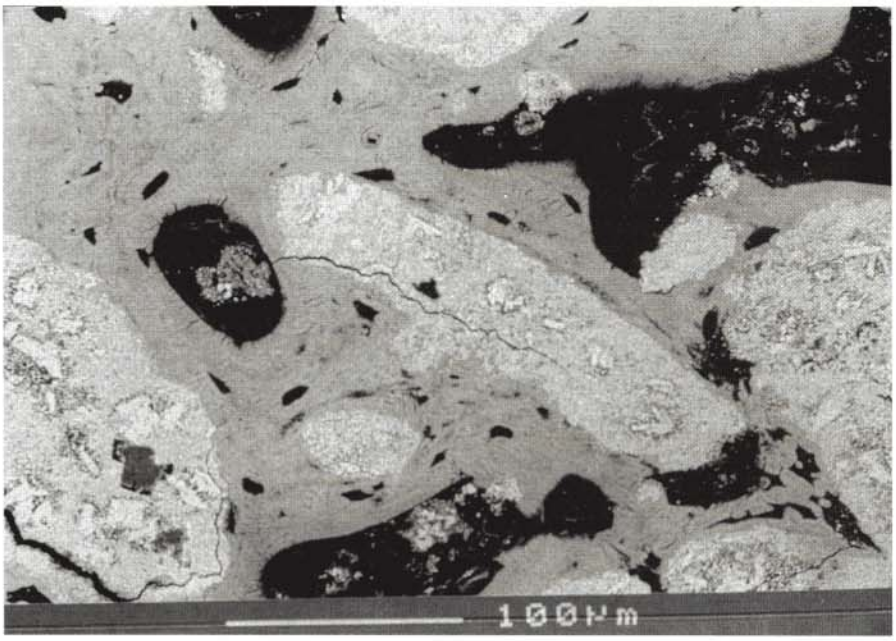


**Figure 3.** Higher magnification showing the lamellar bone closely associated with the BCP granules (original magnification ×320).

Current studies are in progress for dental applications, in particular, for root filling and dental pulp capping.

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