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Research Article

Scaffold Effects of Microporous Biphasic Calcium Phosphate Granules and Role of HPMC Hydrogels in Injectable Multiphasic Bone Substitute Developments

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Abstract Calcium phosphate granules associated with hydrogels were developed as bone substitutes for various clinical applications. These Injectable Bone Substitutes (IBS) should regenerate bone tissue. The efficiency of these multiphasic materials was due to the osteogenic properties of the Microporous Biphasic Calcium phosphate (BCP) granules. The hydrosoluble polymers associated to BCP were considered as a carrier alone. In this study we used hydrosoluble polymers with and without gelling properties in association with the BCP granules. The two suspensions were implanted in femoral epiphysis defects in rabbits and dogs. No foreign body reaction was observed in all samples. However, due to the higher density of the hydrogel/BCP composite caused by the gelling; cell colonization and bone tissue ingrowth was delayed. This study demonstrated that the gelling properties delayed on time biological colonization and decreased the rate of resorption.

Keywords calcium phosphate bioceramics; injectable bone substitute; hydrogels; bone ingrowth; animal models

1 Introduction

Calcium phosphate bioceramics granules associated to hydrosoluble polymers were developed as bone substitutes for various maxillofacial and orthopaedic applications [1]. These Injectable Bone Substitutes should support and regenerate bone tissue and were resorbed after implantation. The efficiency of these multiphasic materials was due to the osteogenic and osteoconductive scaffold properties of the Microporous Biphasic Calcium phosphate. The hydrosoluble polymers associated were considered to be only a carrier to achieve rheological properties and to be a carrier alone for the granules [3,4]. The aim of this study was to demonstrate that the nature of the polymer may interact with the scaffold property of the BCP granules.

2 Materials and methods

We have used 2 hemisynthetic hydrosoluble polymers of polysaccharidic origin. The Hydroxy propyl methyl cellulose (HPMC) with and without silane were combined with microporous BCP granules (mixture of HA and β-TCP solid phase). Silane grafting on HPMC [7] was used as gelling agent of the suspension. The 2 IBS used (without gelling property MBCP Gel™, with gelling IBS2) were implanted in rabbits and dogs in critical size femoral epiphysis defects, and in lumbar muscles. HPMC and HPMC-Si alone without granules was used as control.

All animal handling and surgical procedures were conducted according to European Community guidelines for the care and use of laboratory animals (DE 86/609/CEE) and approved by the local Veterinary School ethical committee.

Eighty female adult New Zealand White rabbits were used. Three groups were constituted IBS1, IBS2, HPMC Si alone. A cylindrical bone defect (6 mm diameter and 8 mm depth) was created at the distal femoral end. The defects were filled with one of the composites carefully compacted to prevent the formation of dead spaces. Intramuscular paravertebral implantations were realized with the 3 implants. The animals were sacrified 6 and 12 weeks after implantation.

Eight adult female Beagle dogs were used in this study. Critical size defect of 8 mm in diameter and 10 mm depth were realized in femoral epiphysis. The defects were filled using the IBS2. IBS2 was prepared under sterile conditions. After 2, and 6 months, after surgery, the animals were euthanised.

Light microscopy, SEM using BSE and image analysis was performed on sections after PMMA embedding.

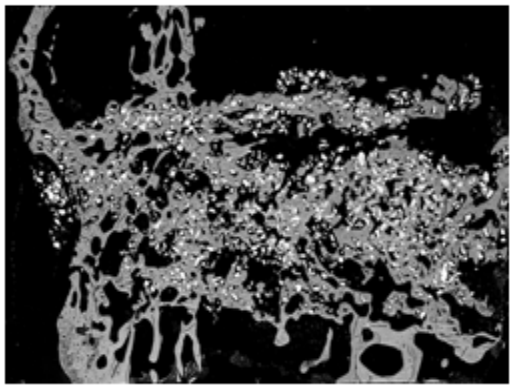

Figure 1: MBCP Gel, rabbit 6 weeks, SEM.

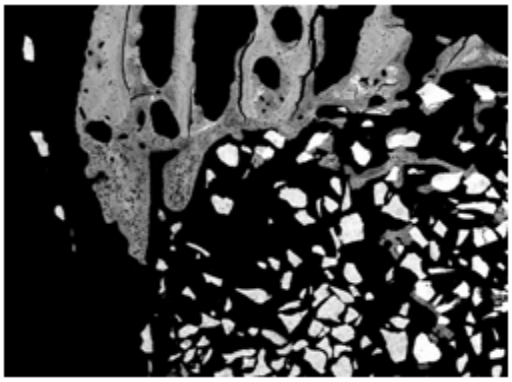

Figure 3: MBCP Gel, dog 8 weeks, SEM.

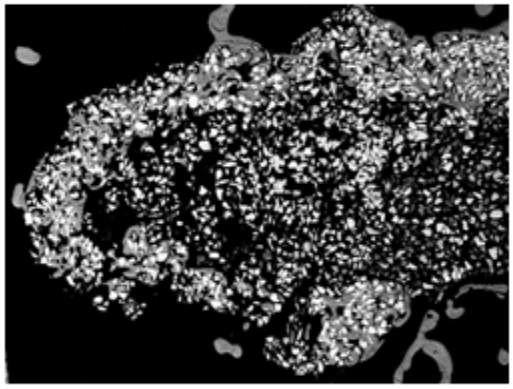

Figure 2: IBS2, rabbit 12 weeks, SEM.

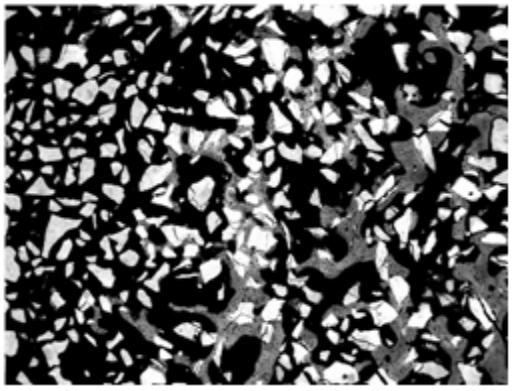

Figure 4: MBCP Gel, dog 24 weeks, SEM.

3 Results and discussion

The first generation of injectable bone substitute IBS1 (MBCP Gel™, Biomatlante SAS) is a non hardening injectable biomaterial. It consists of BCP granules in suspension associated with a hydrosoluble polymer. MBCP Gel™requires rheological properties capable of ensuring bonding of the mineral phase *in situ* with good cell permeability. Bone cells are able to invade the spaces created by the disappearance of the polymer carrier. Bone ingrowth takes place all around the granules at the expense of the resorption of the BCP granules.

The second type of injectable bone substitute (IBS2) is a self-hardening composite. The BCP granules are associated with the silanised hydrogel, HPMC-Si. The hardening of the hydrogel is the result of a cross-linking reaction between silane groups bound to the cellulosic polymer. Prior to cross-linking, the composite is an injectable viscous liquid that hardens in the bone defect, forming a gel loaded with BCP ceramic particles. IBS2 can entirely fill and remain in bone defects.

The *in vivo* results demonstrated the resorption of a part of BCP granules and theirs osteoconductive effects. Bone ingrowth was observed at the expense of the 2 IBS. However faster bone ingrowth was observed for HPMC without reticulation (Figure 1) compared to HPMC Si (Figure 2).

For both materials, we have not observed foreign body reaction. Only during the first three days of implantation some higher sign of inflammation of the skin was observed for the low purified IBS2 probably due to low purification without dialysis and subsequent residual free silane. After 3 days no difference will be observed. Histomorphometrics data obtained from SEM images using BSE were reported on Table 1.

The density of dog trabecular bone epiphysis was 29%.

In SEM we observed low osteoconduction and bone ingrowth in the core of the implant at 8 weeks (Figure 3), and higher bone content and bone trabeculae at 6 months. However limited direct bone contact with the BCP granules are observed (Figure 4) traducing the limited osteoconduction due to the density of the HPMC Si composite.

In polarized light microscopy we note that diameter of the defect was not increased or reduced after 2 months. Only on the surface and in the deep of the defect, between the

	Time implantation	% Granules	% Newly formed bone	% Soft tissue
MBCP Gel rabbit	6 weeks	21 ± 18	29 ± 9	50 ± 13
	12 weeks	15 ± 16	39 ± 5	45.6 ± 5
IBS2 rabbit	6 weeks	30 ± 1	9 ± 9	41 ± 23
	12 weeks	21.5 ± 1.5	8.6 ± 4.1	70 ± 3
HPMC Si alone rabbit	6 weeks	—	1 ± 1	99 ± 1
	12 weeks	—	2 ± 1.5	98 ± 2
IBS2 dogs	8 weeks	33 ± 9	7 ± 5	60 ± 16
	24 weeks	9 ± 11	27 ± 4	64 ± 10

Table 1: Histomorphometric data from BSE images.

granules appears some colonization by bone trabeculae. The SEM using BSE, confirm the lack of mineralization of the fibrillar matrix observed in polarized microscopy between the granules in IBS2.

Movats of HE staining, demonstrate that the spaces between the granules are occupied by a dense fibrillar matrix with a rich cellular content mainly represented by macrophages.

After 24 weeks, the defect was entirely healed. Six samples on 8 implanted indicate a very large resorption of the IBS2, replaced by bone trabeculae similar to the original bone (Table 1). The bone architecture began to be regenerated (Figure 4). It remains residual not resorbed granules between the bone trabeculae. In muscle implantation, there is higher cell colonization and resorption for MBCP Gel compared to IBS2. Numerous multinucleated giant cells are involved in the resorption. Large area with HPMC Si was observed without cell colonization contrarily to HPMC-BCP (MBCP Gel) without reticulation. No fibrous encapsulation or foreign body reaction were observed in both samples.

To date, several injectable biomaterials have been developed. These injectable bone substitutes are made generally of CaP hydraulic cement that hardens in the bone defect. Others are composed of CaP granules suspended in hydrogel.

The biocompatibility and no foreign body reaction are recognized properties of hydrogels based on HPMC [3]. The use of silane for cross-linking improves the rheological and mechanical stability of the injectable bone substitute [5], preventing washout in biological fluid. It is nevertheless recommended that dialysis high purification methods be used. In spite of the fact that there was no dialysis in this study, we did not have any reported allergic, high inflammation reactions in either the muscular area or the bony site of our IBS2 preparation. We observed some difference with a previous study about biological colonisation [6]. The difference in bone ingrowth can be due the interaction and steam effect of the mixture with the medical devices used in the industrial process and the extemporaneous preparation realized in the previous study [6]. A strong interaction has been reported between the HPMC and BCP surface [2] at the unit cell level with HPO_4 integration into the lattice. In this study, before implantation Hr TEM showed strong surface interaction between the HA/TCP granules and the polymer. XRD and FTIR demonstrate a change on time of the ratio HA/TCP (higher HA after a couple of weeks). These interactions at the nanoscale surface may have an influence on osteoconduction and osteogenic properties. The surface of the calcium phosphate and gelling before blood diffusion, delay osteoconduction and cell and tissue colonisation over time at the expense of the composite. Further experiments are required to determine the BCP surface interactions with HPMC Si at the unit cell crystal level.

4 Conclusions

For the initial mechanical stability, prevention of washing out, and initial mechanical properties it will be recommended to have self hardening or reticulation for injectible bioceramics. We need setting/cross-linking, but as a result we had delayed resorption and bone ingrowth.

For closed defect cavities, implant resorption and fast bone ingrowth with secondary mechanical properties, we need a hydrosoluble polymer, without cross-linking.

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