J Mater Sci: Mater Med (2006) 17:1145–1152
DOI 10.1007/s10856-006-0542-7

Injectable bone substitute to preserve alveolar ridge resorption after tooth extraction: A study in dog

D. Boix · P. Weiss · O. Gauthier · J. Guicheux · J.-M. Bouler · P. Pilet · G. Daculsi · G. Grimandi

Received: 8 February 2005 / Accepted: 1 March 2006

Abstract The aim of the present study was to assess the efficacy of a ready-to-use injectable bone substitute on the prevention of alveolar ridge resorption after tooth extraction. Maxillary and mandibular premolars were extracted from 3 Beagle dogs with preservation of alveolar bone. Thereafter, distal sockets were filled with an injectable bone substitute (IBS), obtained by combining a polymer solution and granules of a biphasic calcium phosphate (BCP) ceramic. As a control, the mesial sockets were left unfilled. After a 3 months healing period, specimens were removed and prepared for histomorphometric evaluation with image analysis. Histomorphometric study allowed to measure the mean and the maximal heights of alveolar crest modifications. Results always showed an alveolar bone resorption in unfilled sockets. Resorption in filled maxillary sites was significantly lower than in control sites. Interestingly, an alveolar ridge augmentation was measured in mandibular filled sockets including 30% of newly-formed bone. It was concluded that an injectable bone substitute composed of a polymeric carrier and calcium phosphate can significantly increase alveolar ridge preservation after tooth extraction.

D. Boix · P. Weiss (✉) · J. Guicheux · J.-M. Bouler · G. Daculsi · G. Grimandi
INSERM, U791, Laboratoire dingénierie Ostéo-articulaire et dentaire, LIOAD, 1 place A. Ricordeau, Nantes, F-44042, France
e-mail: pweiss@sante.univ-nantes.fr

O. Gauthier
INSERM EM 99-03 Research center on materials with biological interest, 1 place Alexis Ricordeau, 44042 Nantes cedex 1, France; Surgery Department, National veterinary school, 44307 Nantes cedex 03, France

P. Pilet
Electron microscopy center, University hospital, 1 place Alexis Ricordeau, 44042 Nantes cedex 1, France

Introduction

Following tooth extraction, a blood clot develops into the socket and finally leads to new-bone formation [1]. However, this healing process never allows an *ad integrum* restitution of the initial alveolar bone volume because a physiological resorption systematically conduct to a decrease of height and width of alveolar ridge [2, 3]. The resulting alveolar bone loss may generate difficulties to ensure long-term stability of conventional or implant-borne prosthetic restoration for partially or totally toothless patients [4]. Therefore, various techniques have been proposed to limit alveolar bone loss, such as atraumatic extraction [5], immediate postextraction removable prosthesis [6], immediate placement of dental implant [7, 8] or immediate bone filling of extraction socket [9–11]. Among the numerous available filling materials used to prevent alveolar crest resorption [12–14], allografts and xenografts have shown limits because they sometimes generate problems of supply and risks of crossed contamination [15, 16]. To overcome these limits, many synthetic bone substitutes were proposed among which biphasic calcium phosphates (BCP), associations of hydroxyapatite (HA) and beta-tricalcium phosphate (ß-TCP) [17, 18] Calcium phosphates offer a great potential for bone regeneration since they have chemical composition close to the biological apatite of bone tissues [19]. BCP is generally prepared as blocks, granules or powders and has already proven its efficiency as a bone substitution material in different human applications [20–22]. In addition, previous studies showed that the granulated form of calcium phosphate ceramics gives satisfactory results for alveolar bone regeneration [23, 24]. However, particles present difficulties for placement into exiguous sites such as dental sockets. In order to improve their handling, BCP granules have been associated to a high viscosity polymer solution to form a ready-to-use injectable bone substitute

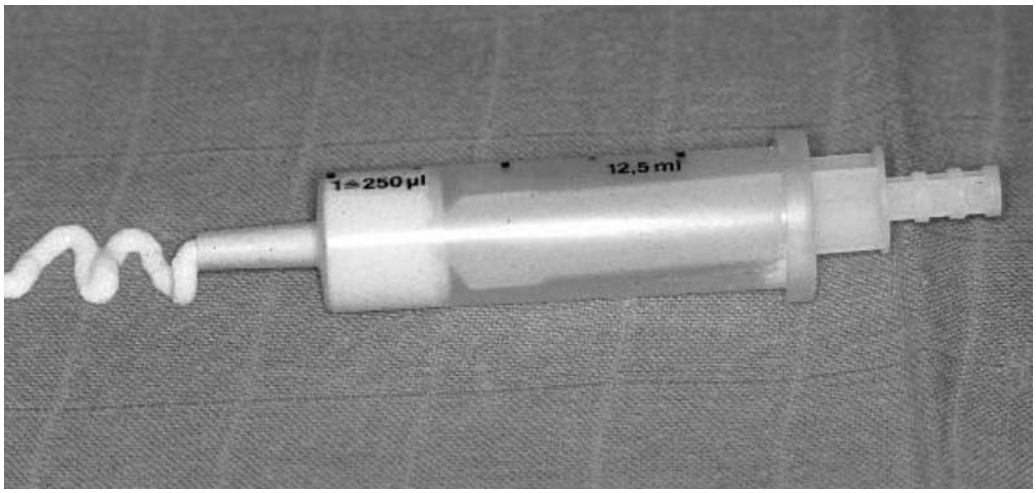

Fig. 1 Photograph of the ready-to-use injectable bone substitute (IBS) placed into an injector

(IBS) [25–28]. Previous animal studies conducted in our laboratory have clearly demonstrated the biocompatibility and the osseoconductive properties of IBS [29–31]. In the present work, we investigated the biofonctionnality and the influence of IBS on alveolar ridge preservation after tooth extraction. A comparative histomorphometric study was performed on fresh extraction sockets. Distal sockets were filled by IBS. As a control, mesial sockets were left unfilled to observe a physiological healing process.

Materials and methods

Injectable bone substitute

The biomaterial used in this study was a composite combining an hydrophilic polymer in solution and a calcium phosphate mineral phase [25, 28, 32, 33].

Polymeric phase

The polymeric phase was a cellulose derivative (hydroxypropylmethylcellulose = HPMC Benecel® MP824, Aqualon, Rueil-Malmaison, France) provided as dry powder. A solution of 2% HPMC was prepared by dissolving raw dry HPMC powder in sterile bi-distilled water under stirring for 3 days at 20°C.

Mineral phase

IBS mineral phase, composed of BCP granules with a 60/40 HA/ ß-TCP weight ratio, was obtained by alcaline hydrolysis of commercial dicalcium phosphate dihydrate. The resulting precipitated was dried to obtain a calcium deficient apatite powder [34]. Thereafter, this powder was humidified to realise a wet granulation (sifters 200–500 μm). Finally, powders have been transformed into ceramic after sintering at 1,150°C. BCP granulometry, alone and in association with HPMC, was controlled with a laser diffraction granulometer (LS230, Coulther, Miami, USA).

Composite : IBS

IBS was finally obtained by mixing the 2% HPMC solution with BCP granules in a 50/50 weight ratio. Thereafter, IBS was put into ready-to-use injectors (Merck Eurolab, Fontenay-sous-Bois, France) (Fig. 1), and sterilised by steam at 121°C for 21 minutes (Kavoclave, Kavo, Warthausen, Germany). The diameter of the cannula was 2 mm. Before implantation, rheologic properties of IBS were controlled with viscosity analyser (DV-I+, Brookfield, Middleboro, USA) and texture analyser (TA TX2, Rheo, Champlan, France).

Animal experiments

Surgical procedure

Three beagle dogs were used for animal experiments. They were 4-year old and respectively weighted 12, 13 and 15 kg. Animals were bred for biomedical studies and kept at the National Veterinary School of Nantes according to European Community guidelines for the care and use of laboratory animals (DE/86/609/CEE). Vaccine calendars of animals were checked and quarantine was installed after animal receptions. Teeth were scaled and polished 3 days before implantations.

General anaesthesia was performed with 12.5 mg/kg intravenous sodium thiopental (Nesdonal, Merial, Lyon, France) followed by volatile anaesthesia with halotane (1%). During surgery, animals received lactated Ringer's solution

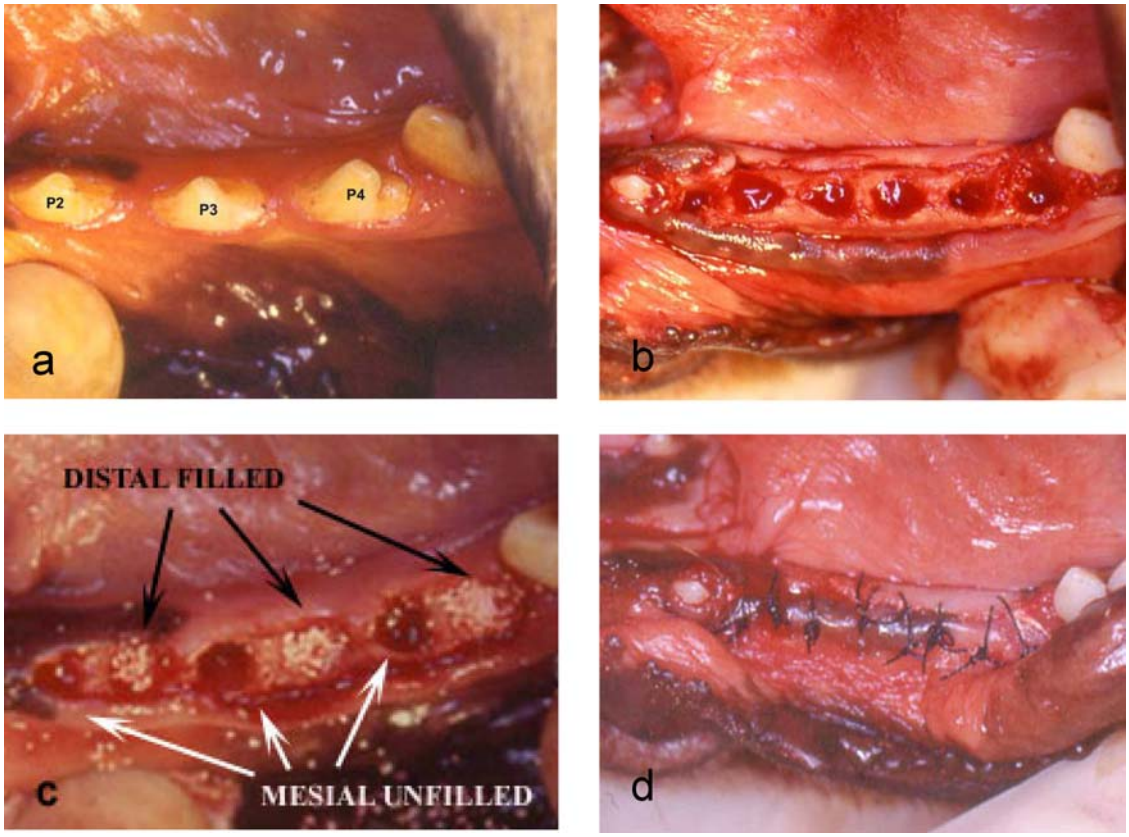

Fig. 2 Photographs of the surgical procedures in Beagle dogs. (a) second (P2), third (P3) and fourth (P4) left mandibular premolars (preoperative view), (b) debrided mesial and distal sockets after second, third and fourth premolar extractions, (c) IBS-filled distal sockets (DS). As control, mesial socket (MS) were left unfilled, (d) overlapping flaps sutures

intravenously (15 to 20 ml/kg/h) and 1g of cephalosporin (Cefaloject, Bristol Laboratories, Paris, France).

For each animal, 6 mandibular premolar teeth (Fig. 2a), and 4 maxillary premolar teeth were extracted. Animal experiments were performed on 36 mandibular sockets and 24 maxillary sockets (dog premolars present 2 roots: 1 mesial + 1 distal).

Sulcular incisions were performed mesially from the canine and distally to the molar and full-thickness buccal and lingual muccoperiosteal flaps were raised. Thereafter, vertical interradicular sections were conducted before each root was mobilised and extracted. All alveolar sites were checked and debrided with a surgical curette (Fig. 2b). After saline solution irrigation, alveolar fillings were performed with IBS in distal sockets only (Fig. 2c). As control mesial sockets were left unfilled. Finally, overlapping hermetic sutures (Mersuture® Dec 2, Ethicon, Jansen-Cilag, Issy-les-Moulineaux, France) were performed to protect operative sites (Fig. 2d).

Antibiotic treatment (cephalosporin, 30 mg/kg/j) was continued for 48 hours after surgery by intramuscular injection. Animals were checked daily and a soft consistency diet was given for 15 days. Sutures were removed 3 weeks after implantation. Then, a normal diet and an every two-days control were performed till sacrifice. Animals were sacrificed 13 weeks after implantation by intravenous injection (15 ml) of overdosed sodium pentobarbital (Dolethal, Vetoquinol laboratory, Lure, France).

Sample preparation

After sacrifice, mandibular and maxillary explants were immediately dissected and fixed in 4% paraformaldehyde solution. Sockets were individually separated with a diamond saw. Samples were dehydrated in graded ethanol (80°, 95°, 100° at 4°C) during 3 days and placed in pure acetone for 24 hours at 4°C. Thereafter, two 8-days embeddments were performed in glycolmethylmethacrylate resin (GMMA) at – 20°C. Finally, inclusions were performed in GMMA (4 days at + 4°C).

Histological evaluation

GMMA resin blocks samples were cut on a frontal level with a hard tissue microtome (Reichert-Jung 2050, Cambridge instruments, Vienna, Austria). The resulting surfaces were coated with gold-palladium metallisor (AE1230, EMScope,

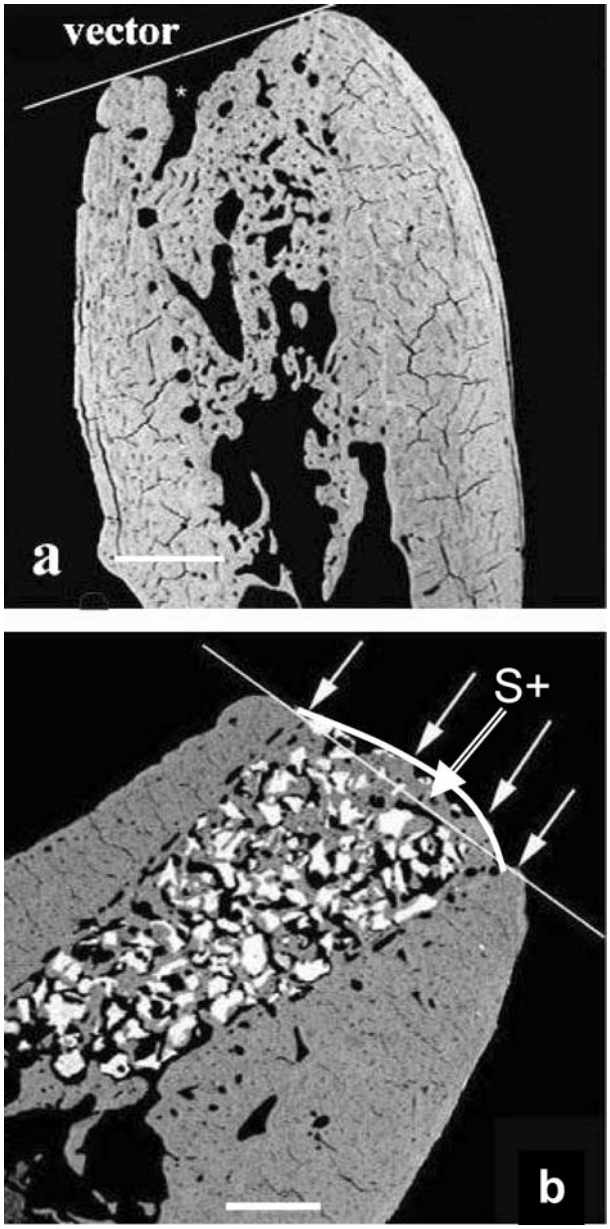

Fig. 3 Scanning electron microscopy observations of axial sections of mandibular sockets after three months of healing. With backscattered electrons newly formed bone appeared in dark grey, BCP ceramic in light grey and soft tissues and bone marrow in black (a, b). A tangent vector [white line] to the vestibular and lingual cortical was drawn. (bar = 0,5 mm). (a) unfilled socket. Note the presence of a collapse below the vector (*), (b) IBS-filled socket. Note the presence of mineralized tissues above the vector (white arrows). S+ indicates the alveolar ridge augmentation above the vector (white double arrows)

Ashford, UK) and analyzed in SEM (JSM 6300, Jeol, Tokyo, Japan) using backscattered electrons (BSE) at 15 kV.

Histomorphometric study

SEM observations were digitalised and binarised in order to carry out a semi-automatic image analysis (PC Quantimeter 500MC, Leica, Cambridge, UK). The outline of each socket was identified and a tangent vector to the vestibular and lingual corticals was drawn (Fig. 3). Thereafter, image analysis allowed to measure the mean heights (MeH) and the maximal heights (MxH) that separated the vector to the summit of alveolar crest. These heights were negatively reported when alveolar crest forms a concavity with regard to vector (Fig. 3a) and positively report in case of convex relief (Fig. 3b).

When MeH and MxH were positive, it was possible to identify a surface (S+) above the vector (Fig. 3b). First of all, S+ was expressed as percentage of the total socket surface (TS). Secondly, the components of S+ (artificial colours in the final computerized images indicate the different tissue component surfaces: BCP ceramic particles = White, newly-formed bone = grey and non-mineralized soft tissues = black (Fig. 3b) were identified and measured with image analysis. Finally, surfaces of the three different components were expressed as a percentage of S+.

Statistical analysis

Three observations were successively performed at 1/4 mesial, in the middle and at 1/4 distal of each socket. Seven sockets were voluntarily eliminated from the study because of complications during surgery. Results were expressed as mean (±SEM) of MeH and MxH. Comparative studies of means of the 48 filled mandibular and the 48 unfilled mandibular sections, as well as the 33 filled maxillary and the 30 unfilled maxillary observations were performed using one-way analysis of variance (ANOVA) followed by a post-hoc test (Fisher's projected least significant difference) with a significance value of $p < 0,01$.

Results

Biomaterial characterization

IBS granulometry

Laser diffraction showed that BCP granule diameters ranged from 200 to 500 μm. This class of particles represents 78% of the total volume of BCP before incorporation in HPMC polymer, 70% in the composite before sterilisation, 66% in the composite after sterilisation, 65% in the composite at the beginning of injection and 64% in the composite at the end of the injection (Fig. 4).

IBS rheologic properties

The composite viscosity was *1,950,000 mPa.s.* and a strength ranging from 200 to 600 g was necessary to inject IBS (Fig. 5). This force allows a not forced injection of the product

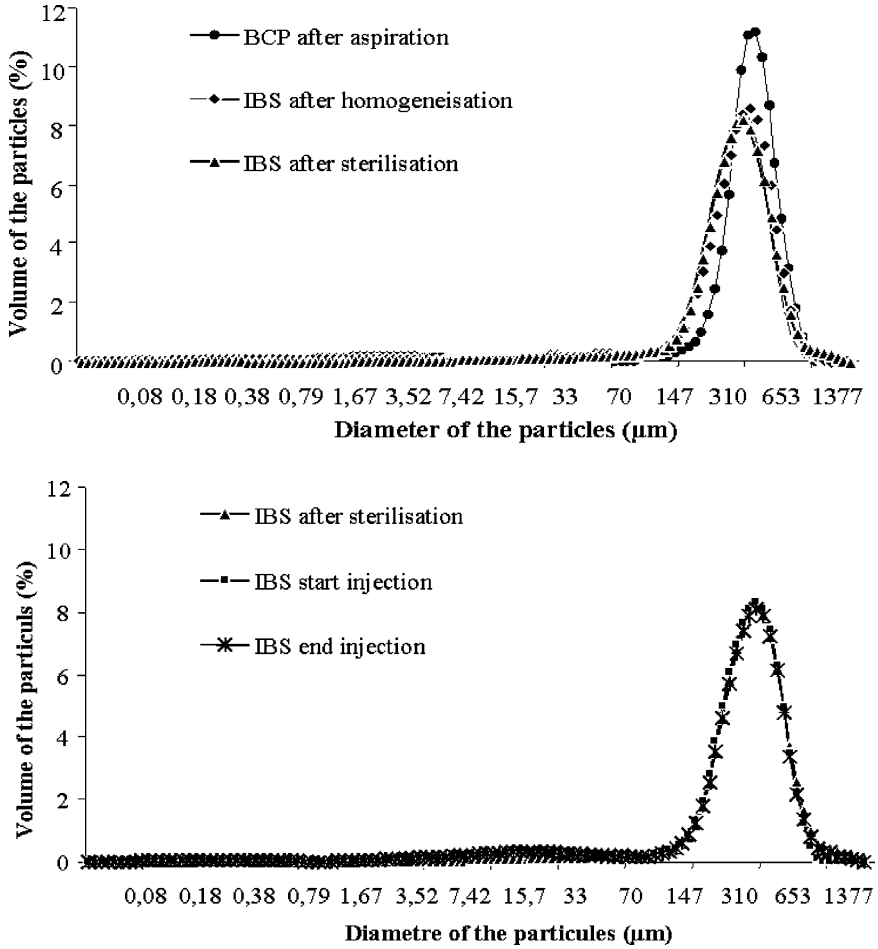

Fig. 4 Mean Particle size distribution of BCP powder, BCP in IBS before sterilisation and in the final injected product. Results were obtained by laser diffraction and are expressed as percent of the total volume of particles

which remains homogeneous for displacements of the piston lower than 15 mm. For displacements of the piston higher than 15 mm, the force necessary to the biomaterial extrusion increases quickly to reach 5,6 kg (data not shown). Thereafter, injection becomes difficult then impossible with demixtion of composite.

Clinical results

Minor complications were observed during the surgery. One oro-antral fistula was observed. In addition, 2 vestibular process and 4 dental roots were fractured. These 7 sockets (3 maxillary and 4 mandibular) were not retained for further study. No infectious complication happened during the postoperative phase. Sutures were removed after a 3 weeks healing period, without any gingival inflammation.

SEM evaluations

No BCP granules were found in unfilled sites indicating the absence of material migration from the filled sites to the control sites.

For all unfilled sockets, crest collapse was always observed. Alveolar crest presented a morphology forming a concavity with relation to the cortical tangent vector (S−). No mineral component was detected between alveolar crest and vector (Fig. 3a).

In filled sockets, results were different for maxillary and mandibular sites. In filled and unfilled maxillary sockets, slight collapse crest has been systematically observed, but the bone loss appeared less important with IBS. In most of filled mandibular sockets, crest contour presented a convexity with respect to the vector.

Quantitative evaluation

In unfilled sites, there is no significant difference of mean (MeH) and maximal (MxH) height values between maxillary and mandibular sites (Fig. 6). However, IBS induce a significant decrease of alveolar crest resorption in the maxillary sockets, respectively 50% for MeH and 30% for MxH. Interestingly, IBS allowed MeH and MxH to reach positive values in mandibular filled sockets with the expression of S+ representing 6% of the total socket surface.

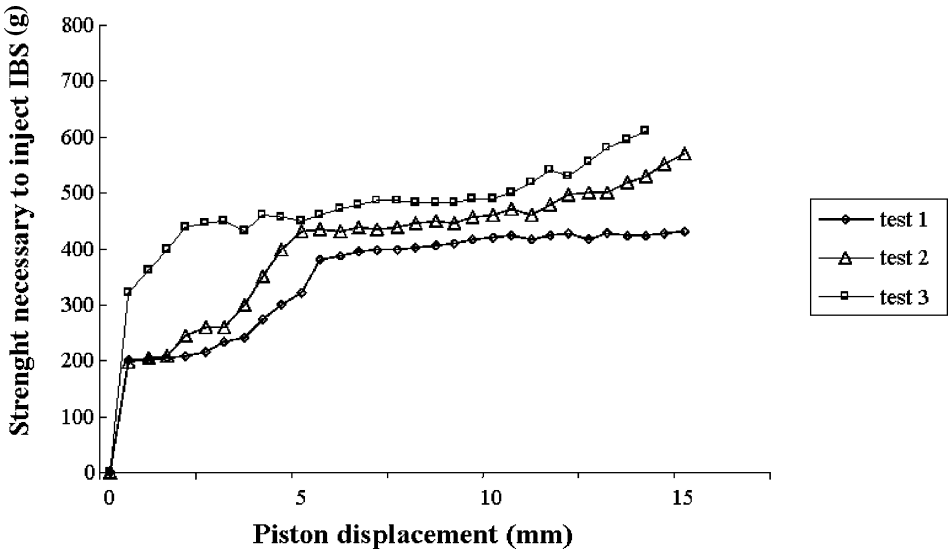

Fig. 5 Extrusion of the BCP mixture through a 2-mm hole at a rate of 0.1 mm/sec. Compression strength measurements on the piston of an injection syringe were recorded with a computer to obtain these profiles. Experiments was repeated three times (test 1, 2 and 3)

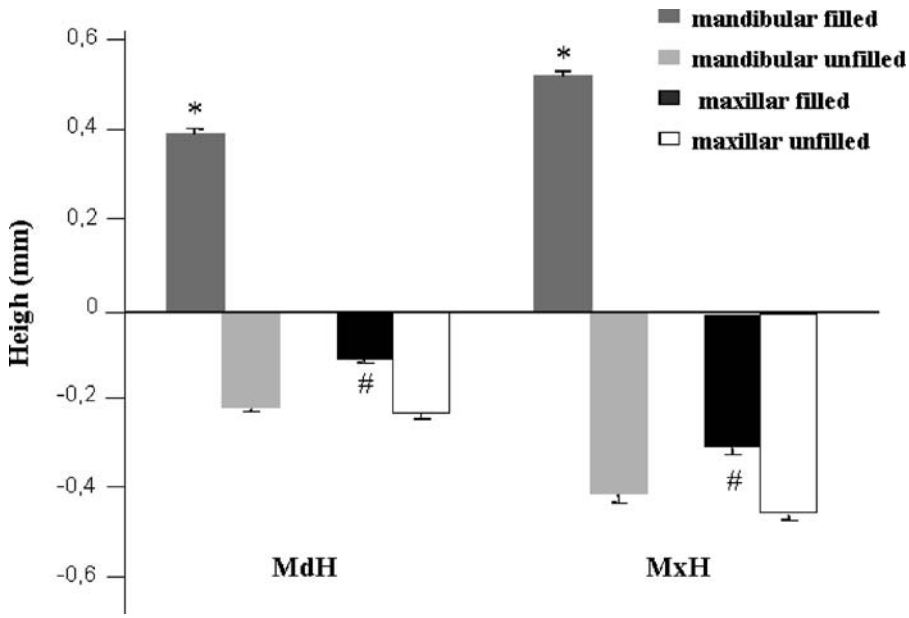

Fig. 6 Variations of mean heights (MeH) and maximal heights (MxH) that separated the vector of the alveolar crest summit after three months of socket healing either in control filled maxillary or mandibular sites. Results are expressed in mm [mean ± SEM]. $(*)P < 0,01$ as compared to maxillary control, (#) $p < 0,01$ as compared to mandibular control

Non-mineralised areas occupy 55.30% ±10.79 of the surface above the vector (S+). In addition, newly-formed bone and BCP represent respectively 29.90% ± 8.92 and 14.90% ± 5.25 of S+.

Discussion

In the present work, we have shown for the first time that an injectable biomaterial, composed of a biphasic calcium phosphate and a cellulose derivative polymer, could prevent alveolar crest resorption after tooth extraction.

Systematic filling of a non-damaged extraction socket is not justified because dental surgeons agree that the physiologic blood clot is one of the best healing material [1]. Nevertheless, after trauma or destructive periodontal disease, and in cases of thin alveolar bony-walls with high sensibility to postextractional resorption, this physiological healing process improved difficulties to support conventional or implant-supported prosthetic restoration [35, 36]. Furthermore, aesthetic and functional requirements from the patients are more and more important and can not be fulfilled without a sufficient bone volume [37]. To prevent the alveolar bone crest resorption, numerous filling biomaterials have been used among which calcium phosphate ceramics: hydroxyapatite (HA) and beta-tricalcium phosphate (ß-TCP) which have respectively shown variable results [38–40]. To improve HA and ß-TCP physicochemical properties, biphasic calcium phosphate (BCP), an HA/ß-TCP association, were secondly proposed [19]. When HA, ß-TCP or BCP were used

for alveolar filling, the best resorption/substitution process were reported with granulated form [23, 24]. To overcome the manipulation limits of particles into exiguous tooth sockets, we have recently proposed to associate BCP granules with a polymer solution to provide a ready-to-use injectable bone substitute (IBS). [25, 26, 28]. The osteoconductive potential of this innovative biomaterial was previously demonstrated for clinical application in our laboratory [29–30] and in the same animal model [31] with the quantification of each component, BCP, bone and soft tissue. In an attempt to prevent the bone crest resorption after tooth extraction, the present study investigates the efficiency of IBS to promote the preservation of the alveolar ridge morphology after filling of fresh extraction sockets.

In a first set of experiments, we sought to confirm whether IBS exhibit handling properties compatible with its clinical potential applications as a socket filling material. Controls performed before implantation confirmed that IBS presents rheologic properties allowing to obtain a homogeneous filling of dental sockets. Injection from the bottom to the top of the socket did not reveal any difficulties. However, the limit in the increase of granule size is the preservation of IBS rheologic properties and the 200 to 500 μm particles seems to be the higher diameter appropriate to alveolar filling. The homogenisation triggers a reduction of 8% of calcium phosphate mean size particles. However, BCP granulometry in the final product remains in the range of 200–500 μm. To strengthen our preliminary data, we are currently conducting studies on IBS with 40 to 80 μm and 80 to 200 μm diameter BCP granules.

The main objective of this work was to obtain quantitative results on alveolar bone crest resorption. Among the numerous available tools, we choosed the semi-automatic image analysis because it allows to analyse quickly and accurately a large amount of informations [30, 41]. However, our quantitative data showed some limitations since they were carried out with respect to the vestibular and lingual process, which may have been subjected to a post-extraction resorption process. Consequently, previous studies carried out on the alveolar resorption after dental extraction generally start from a fixed point such as an adjacent tooth or a dental implant [42–45] Nevertheless, this work highlights the clinical benefice provided by IBS. Most of the previous filling studies performed on Beagle dogs [46–48] have only used mandibular sockets, because of the extreme difficulty to atraumatically extracted maxillary teeth. In the present work, six mandibular premolar teeth and four maxillary premolar teeth were used for each animal to increase the available sockets for statistical analysis. Nevertheless, the study of the superior and inferior sockets was voluntarily separated in order to evaluate a potential difference between these two sites. To our knowledge, no documented research exists to explain our higher results obtained with mandibular sockets. Among the explaining causes are: (i) a larger cortical thickness to the mandible, (ii) a major quantity of injected material in mandibular sites and finally, (iii) a greater difficulty to carry out histological sections in the maxillary.

In the filled mandibular sockets, analysis of components of S+ has shown that non-mineralised areas occupied the half surface of the crest height gain. Two hypothesis can be assumed on the future of S+. First of all, since non-mineralised areas are located at the periphery of BCP and newly-formed bone, one can think that after BCP substitution, bone healing process will lead to the reduction of these non-mineralised areas. One can also assume that BCP and newly-formed bone only help on a temporary time the convex crest morphology maintenance. Secondary resorption would then be only delayed. Between both hypothesis, it is wise to envisage an intermediary alternative for which alveolar filling would enable, not a height gain, but an absence of resorption after complete bone healing and BCP resorption/substitution process. This alternative would constitute an interesting overhang for conventional or implant-borne prosthetic rehabilitations. However, this hypothesis is to be confirmed by further experimentations over longer periods.

Conclusion

This study demonstrates that a composite biomaterial prevents alveolar crest resorption after tooth extraction. The increasing number of partially or completely edentulous patients is in close relationship with population aging process. Therefore, more comfortable and durable prosthetic rehabilitations are a great challenge for the scientific community. In addition, the approval of alveolar bone preservation biomaterials, such as IBS, could make possible the development of candidates to oral implantology. In this context, our data open new therapeutic windows for pre-implant surgery in unfavourable anatomic situations. IBS could be further proposed for human clinical applications such as reconstruction of osseous deficiencies, peri-implantitis treatment or sinus floor elevations.

Acknowledgment The authors gratefully acknowledge Ms Anne Gouyette for his helpful contribution to laser diffraction analysis and Ms Karine Sinander for critical reading of this manuscript. This work was supported by "Contrat de Plan Etat-Région Pays de Loire. Axe biomatériaux S3".

References

1. M. H. AMLER, *Oral Surg. Oral Med. Oral Pathol.* **27** (1969) 309.
2. T. KARRING, N. P. LANG and J. LINDHE, Clinical Periodontology and Implant Dentistry (Blackwell Publishing, Oxford, 2003).

3. L. JAHANGIRI, H. DEVLIN, K. TING and I. NISHIMURA, *J. Prosthet. Dent.* **80** (1998) 224.
4. T. H. HOWELL, J. FIORELLINI, A. JONES, M. ALDER, P. NUMMIKOSKI, M. LAZARO, L. LILLY and D. COCHRAN, *Int. J. Periodontics Restorative Dent.* **17** (1997) 124.
5. J. S. SEIBERT, *Dent. Clin. North Am.* **37** (1993) 265.
6. G. S. WEINTRAUB, *Dent. Clin. North Am.* **33** (1989) 399.
7. L. F. COOPER, A. RAHMAN, J. MORIARTY, N. CHAFFEE and D. SACCO, *Int. J. Oral Maxillofac. Implants* **17** (2002) 517.
8. G. L. DOUGLASS and R. L. MERIN, *J. Calif. Dent. Assoc.* **30** (2002) 362.
9. Z. ARTZI, H. TAL and D. DAYAN, *J. Periodontol.* **71** (2000) 1015.
10. H. R. STANLEY, M. B. HALL, A. E. CLARK, C. J. KING, L. L. HENCH and J. J. BERTE, *Int. J. Oral Maxillofac. Implants* **12** (1997) 95.
11. A. BOLOURI, N. HAGHIGHAT and N. FREDERIKSEN, *Compend. Contin. Educ. Dent.* **22** (2001) 955.
12. C. J. DAMIEN and J. R. PARSONS, *J. Appl. Biomater.* **2** (1991) 187.
13. A. N. CRANIN, M. KATZAP, E. DEMIRDJAN and J. LEY, *J. Oral Implantol.* **27** (2001) 43.
14. E. P. BARBOZA, *Int. J. Periodontics Restorative Dent.* **19** (1999) 601.
15. K. A. AL RUHAIMI, *Int. J. Oral Maxillofac. Implants* **16** (2001) 105.
16. L. VASTEL, V. LEMERCIER, L. KERBOULL and M. KERBOULL, *Rev. Chir. Orthop. Reparatrice Appar. Mot.* **85** (1999) 164.
17. R. Z. LEGEROS, J. R. PARSONS, G. DACULSI, F. DRIESSENS, D. LEE, S. T. LIU, S. METSGER, D. PETERSON and M. WALKER, *Ann. N Y Acad. Sci.* **523** (1988) 268.
18. J. M. BOULER, R. Z. LEGEROS and G. DACULSI, *J. Biomed. Mater. Res*. **51** (2000) 680.
19. R. Z. LEGEROS, in "Biomechanics in orthopedics", edited by P. Niwa (Springler-Verlag, Tokyo, 1991), p. 147.
20. G. DACULSI, N. PASSUTI, S. MARTIN, C. DEUDON, R. Z. LEGEROS and S. RAHER, *J. Biomed. Mater. Res* .**24** (1990) 379.
21. E. B. NERY, A. ESLAMI and S. R. VAN, *J. Periodontol.* **61** (1990) 166.
22. A. PIATTELLI, A. SCARANO and C. MANGANO, *Biomaterials* **17** (1996) 1767.
23. M. S. BLOCK and J. N. KENT, *J. Oral Maxillofac. Surg*. **44** (1986) 89.
24. D. J. BELL, *J. Prosthet. Dent.* **56** (1986) 322.
25. G. DACULSI, P. WEISS, J. DELECRIN, G. GRIMANDI and N. PASSUTI. CNRS Patent WO 95/21634: [licence: Biomatlante, MBCP gel^{TM}]. France. (1994).
26. G. DACULSI, R. ROHANIZADEH, P. WEISS and J. M. BOULER, *J. Biomed. Mater. Res*. **50** (2000) 1.
27. X. BOURGES, M. SCHMITT, Y. AMOURIQ, G. DACULSI, G. LEGEAY and P. WEISS, *J. Biomater. Sci. Polym. Ed.* **12** (2001) 573.
28. G. GRIMANDI, P. WEISS, F. MILLOT and G. DACULSI, *J. Biomed. Mater. Res.* **39** (1998) 660.
29. O. GAUTHIER, J. M. BOULER, P. WEISS, J. BOSCO, G. DACULSI and E. AGUADO, *J. Biomed. Mater. Res.* **47** (1999) 28.
30. O. GAUTHIER, J. M. BOULER, E. AGUADO, P. PILET and G. DACULSI, *Biomaterials* **19** (1998) 133.
31. O. GAUTHIER, D. BOIX, G. GRIMANDI, E. AGUADO, J. M. BOULER, P. PILET, P. WEISS and G. DACULSI, *J. Periodontol.* **70** (1998) 359.
32. O. GAUTHIER, I. KHAIROUN, J. BOSCO, L. OBADIA, X. BOURGES, C. RAU, D. MAGNE, J. M. BOULER, E. AGUADO, G. DACULSI and P. WEISS, *J. Biomed. Mater. Res.* **1** (2003) 44.
33. P.-J. WEISS, L. OBADIA, D. MAGNE, X. BOURGES, C. RAU, T. WEITKAMP, I. KHAIROUN, J. M. BOULER, D. CHAPPARD, O. GAUTHIER and G. DACULSI, *Biomaterials* **24** (2003) 4591.
34. J. M. BOULER, M. TRECANT, J. DELECRIN, J. ROYER, N. PASSUTI and G. DACULSI, *J. Biomed. Mater. Res.* **32** (1996) 603.
35. M. E. GHER, G. QUINTERO, J. B. SANDIFER, M. TABACCO and A. C. RICHARDSON, *Int. J. Periodontics. Restorative Dent.* **14** (1994) 332.
36. J. S. SEIBERT and H. SALAMA, *Periodontol. 2000* **11** (1996) 69.
37. R. SHANAMAN, M. R. FILSTEIN and M. J. DANESH-MEYER, *Int. J. Periodontics Restorative Dent.* **21** (2001) 345.
38. J. K. MATHAI, S. CHANDRA, K. V. NAIR and K. K. NAMBIAR, *Aust. Dent. J.* **34** (1989) 421.
39. O. BAHAT, C. DEEB, T. GOLDEN and O. KOMARNYCKIJ, *Int. J. Periodontics Restorative Dent.* **7** (1987) 34.
40. I. M. BROOK, W. SATTAYASANSKUL and D. J. LAMB, *Br. Dent. J.* **164** (1988) 212.
41. J. R. NEFUSSI, A. OLLIVIER, M. OBOEUF and N. FOREST, *Bone* **20** (1997) 5.
42. W. BECKER, S. E. LYNCH, U. LEKHOLM, B. E. BECKER, R. CAFFESSE, K. DONATH and R. SANCHEZ, *J. Periodontol.* **63** (1992) 929.
43. K. S. CHO, S. H. CHOI, K. H. HAN, J. K. CHAI, U. M. WIKESJO and C. K. KIM, *Clin. Oral Implants Res.* **9** (1998) 419.
44. C. K. KIM, K. S. CHO, S. H. CHOI, A. PREWETT and U. M. WIKESJO, *J. Periodontol.* **69** (1998) 26.
45. C. K. KIM, H. Y. KIM, J. K. CHAI, K. S. CHO, I. S. MOON, S. H. CHOI, J. S. SOTTOSANTI and U. M. WIKESJO, *J. Periodontol.* **69** (1998) 982.
46. A. D. SHERER, R. G. SLIGHTER, S. S. ROTHSTEIN and H. P. DROBECK, *J. Prosthet. Dent.* **57** (1987) 331.
47. D. KOHAVI, S. R. POLLACK, G. BRIGHTON and B. BULKIN, *Clin. Oral Implants Res.* **2** (1991) 145.
48. P. J. BOYNE, S. S. ROTHSTEIN, K. I. GUMAER and H. P. DROBECK, *J. Oral Maxillofac. Surg.* **42** (1984) 589.