The safety and efficacy of an injectable bone substitute in dental sockets demonstrated in a human clinical trial

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Abstract

This study is the first report of a clinical evaluation of an injectable bone substitute. This injectable bone substitute was prepared by suspending biphasic calcium phosphate (BCP) particles with diameters ranging between 80-200 µm in a water-soluble cellulose polymer carrier phase. It was used for filling bone defects after tooth extractions in eleven patients. The first objective of the study was to investigate the safety of the filler material. The second objective was to investigate the efficacy of the filler material for filling human tooth sockets by preventing alveolar bone loss. Radiographic density measurements of the surgical sites gradually increased to those of the surrounding host bone. Three years after surgery, small biopsies of the implanted areas were harvested and analyzed by using micro-computed tomography, non-decalcified histology and histomorphometry. The BCP granules appeared in direct contact with mineralized bone tissue, thereby supporting bone growth. A gradual substitution of the filler by bone tissue was observed thus preserving the height of the alveolar bone crest.

Introduction

For more than 25 years, calcium phosphate (CaP) biomaterials have been used [1] in various clinical applications such as for filling bone defects [2], bone augmentation in spinal arthrodesis [3,4], periodontal treatment [5], and as coatings on metal implants [6]. CaP biomaterials are considered to be osteoconductive, achieving coalescence with bone tissue [7] [8]. More recently, new percutaneous techniques using injectable biomaterials have been developed in spinal and orthopedic surgery [9,10]. Acrylic cementation of vertebrae and filling of bone cysts were the first documented applications [11]. Although acrylic cements fulfill the requirements of injectability, filling complex-shaped bone defects and setting very firmly *in situ*, these materials nevertheless lack osteoconductivity and degradability.

Injectable CaP biomaterials should associate efficient bone colonization on implantation with non-invasive surgical techniques. Two types of injectable bone substitutes IBS, are being developed in laboratories. Self-setting Calcium Phosphate Cements (CPC) were the first injectable bone substitute developed for percutaneous applications [12].These hydraulic cements, however, are not ready-to-use, requiring extemporaneous mixing with various setting times *in situ*. Furthermore, most of CPC wash out when they come in contact with body fluids before setting [13]. Additionally, once hardened, CPC produces a dense material with irregular microporosity and are slowly degraded *in vivo* [14,15], whereas numerous studies have shown that interconnected macropores are needed to facilitate bone ingrowth [16]. A second type of injectable bone substitute , consisting of CaP ceramic granules suspended in a water-soluble polymer carrier phase, has been developed in our laboratory [17]. This Injectable Calcium Phosphate Ceramics Suspension (ICPCS) is ready-to-use and osteoconductive, but lacks initial mechanical strength. We have shown in various animal models that bone growth occurred very rapidly due to the material's interconnected macroporosity [18,19].

The present study is the first report on the clinical evaluation of our injectable bone substitute, ICPCS. The injectable biomaterial was used for filling bone defects after tooth extractions in order to prevent alveolar bone loss. This clinical evaluation involving 11 patients was conducted according to European ethical guidelines and French regulations. The first objective of the study was to investigate the safety of ICPCS in clinical use. The second objective was to investigate the efficacy of this material when used to fill intact human tooth sockets together with a gradual substitution of the material by bone tissue. Radiographic density measurements of the surgical sites were performed and compared to those of the surrounding host bone up to 3 years after implantation. Biopsies of the implanted areas were taken and analyzed by using micro-computed tomography, non-decalcified histology and histomorphometry.

Materials and Methods

1. Preparation of the material

As previously published, this injectable bone substitute consisted of CaP ceramic particles suspended in an aqueous polymer solution [20]. Ceramic biphasic CaP particles with diameters of between 80 to 200 µm were sterilized by gamma irradiation (MBCP®, Biomatlante, Vigneux, France). The ceramic was composed of 60% hydroxyapatite (HA) and 40% β-tricalcium phosphate (β-TCP) according to X-ray diffraction and infrared spectroscopy [21,22]. The injectable bone substitute, ICPCS, was prepared by mixing the ceramic particles with a polymer solution (CNRS Patent WO 95/21634). The polymer solution consisted of sterile hydroxypropylmethylcelullose 2% in a saline solution (Ocucoat® , Bausch & Lomb Storz, Clearwater, FL 33759). A ceramic to aqueous polymer solution ratio of 58/42 in weight was used. The clinical batches were prepared aseptically by pharmacists in clean room facilities (class 100) of the University Hospital Center in Nantes. Glass syringes (5 ml, Rothe)

were filled with 2 ml of the paste mixture, sealed with a silicone plug (Vygon™) and packaged. Thus, the clinical batches were sterile, ready-to-use and injectable.

2. Design of the clinical trials

Two protocols were submitted and approved by the local clinical ethics committee (Comité Consultatif de Protection des Personnes dans la Recherche Biomédicale des Pays de la Loire, CCPPRB) under reference numbers BRD99/9C and BRD03/12E. In both studies, the principal investigator and promoter were Dr. Pierre Weiss (DDS, PhD) and the University Hospital Center in Nantes, respectively.

The aim of the first clinical evaluation (BRD99/9C) was to demonstrate the safety of ICPCS when used to fill alveolar bone defects after tooth extractions. Filling the tooth sockets with ICPCS should prevent the resorption of the mandible bone crest, and thus preserve it for possible dental implantology and prosthetics restorations in the future. At the beginning of the study, it was decided to limit the filling of the sockets to the first molars (36 and 46) at the mandible, with indication for extraction, so that local conditions could be both identical and comparable. Out of sixteen eligible candidates, eleven patients signed the formal consent form and were finally included in the study. In these 11 patients, 18 teeth were extracted and alveolar bone defects were immediately filled with 1 too 2 ml of the ICPCS. According to the protocol design, all the patients included (11) were clinically examined immediately (day 0), and at 15 days, 3 months (day 90) and 6 months (day 180) after surgery. Retroalveolar X-ray radiographs were taken at the same time periods. The clinical safety of ICPCS was assessed by checking the absence of a radiolucent line between the biomaterial and the osseous walls of the socket in X-rays. The bioactivity of ICPCS was evaluated by measuring the radiographic densities of the bone at the surgical sites and of the contiguous host bone.

The aim of the second clinical evaluation (BRD03/12E) was to investigate the clinical behavior of ICPCS after it was used to fill the tooth sockets. For this purpose, the eleven patients included in the first clinical evaluation were asked to come for harvesting a biopsy from the surgical site 36 months after implantation. Only 4 patients were found 3 years after surgery. Three patients accepted to sign the formal consent form and were included in the second clinical study. The 3 patients were a 36-year-old woman in case 1, a 42-year-old woman in case 2 and a 22-year-old man in case 3.

3. Surgical procedure

The teeth were extracted from the mandible bone under local anesthesia using a preservative and non-traumatic surgical procedure. Incisions were performed medially and distally in the gum around the tooth. Full-thickness flaps were raised in the periosteal region. The integrity of the bone in the tooth socket was preserved by careful separation of the roots. Thereafter, each root was extracted and the alveolar sites were visually checked and lanced using a surgical curette. The bleeding sockets were rinsed with saline solution. The alveolar sockets were then filled with 1 to 2ml of the ICPCS. Finally, the surgical sites were closed by suturing the gum with biodegradable sutures (Mersuture® Dec 2, Ethicon, Jansen-Cilag, Issy-les-Moulineaux, France). Immediately after surgery, patients were questioned about pain using a self-evaluation scale from 1 to 5.

Three years after surgery, 3 patients out of 11 were found and agreed to provide a biopsy of the ICPCS-filled alveolar sockets, signing the formal consent forms. The biopsies were harvested under local anaesthesia after a full thickness incision in the gingival mucous membrane in order to expose the alveolar crest over the implantation zone. A bone sample of the ICPCS-filled socket about 2 mm in depth and 3.2 mm in diameter was then removed using a needle for bone marrow aspiration/biopsy (Jamshidi™ 10 cm, 8 Ga REF CJ4008X,

Allegiance- Châteaubriant, France). The biopsy was immediately put into a labeled container with the fixative, buffered saline solution at pH 7.2 containing 2% formaldehyde and 2.5% glutaraldehyde.

4. Radiographic assessment and statistical analysis

Retroalveolar X-rays were made using paralleling techniques of radiography devices (Rinn Co., Elgin, Ill) [23]. The apparatus was a long cone generator (ORIX 70, Manufacturer) with the X-ray generator powered at 70 Kvp, 8 my, 560 VA. Five retroalveolar X-rays were taken for each site. X-ray images were taken immediately (day 0), at 15 days, 3 months (day 90) and 6 months (day 180) after surgery. For 3 patients (second protocol), X-rays were taken 3 years after surgery. The X-ray images were independently examined by 3 doctors in dental surgery and their clinical assessments were recorded in a logbook. To determine radiographic density, all X-rays were digitalized and normalized using an image analysis system (Leica Quantimet 4). The black areas in the images (free areas) were used for calibration. The average gray density of the host bone and ICPCS-filled defects was then determined. The relative radiographic density value of the ICPCS -filled defect was expressed as a percentage of that of the host bone.

The radiographic density scores underwent statistical analysis (SYSTAT 10.2 Software, Company Inc.). First, the data were studied using the Lilliefors test that showed a Gaussian distribution of the quantitative variables. Second, repeated measure analyses were performed in order to assess the effect of two parameters, time and subject, and their interaction with the bone density scores. Statistical significance was determined as $p < 0.05$.

5. Histological and histomorphometrical analyses

After fixation for 10 days, the biopsies of the 3 patients were processed for non-decalcified histology. The samples were dehydrated in ascending graded ethanol series (70-100%, 24 h

for each grade) and then in pure acetone for 24 h. Thereafter, the samples were put in glycolmethylmethacrylate resin (GMMA) at –20°C for 8 days. Finally, embedding was performed in GMMA at 4°C for 4 days.

Prior to cutting, the embedded biopsies were characterized using laboratory desktop micro-computed tomography (µCT, SkyScan 1072, Aartselaar, Belgium). The µCT apparatus consisted of the combination of an X-ray shadow microscopic system and tomographic reconstruction software. Typical data collection cycles for reconstruction contain shadow image acquisitions from 200 to 400 views over 180 or 360 degrees of object rotation. The X-ray shadow projections were digitized as 1024x1024 pixels with 4096 brightness gradations (12 bit) for cooled camera or 256 gradations (8 bit). The spatial resolution obtained was 5 µm corresponding to almost $1x10^{-7}$ cubic mm voxel size.

Non-decalcified blocks were also processed for scanning electron microscopy (SEM) observations. The samples were polished and sputtered with a thin layer of gold-palladium (Denton Vacuum, England). Contiguous SEM micrographs were automatically taken using back scattered electrons at 15 kV (BSEM, LEO 1450 VP). Ceramic particles, mineralized bone and non-mineralized tissue were easily distinguished by their respective gray levels using the back scattered electron mode. SEM images were taken at different magnifications.

The blocks were cut into 100 µm slides using a circular diamond saw (microtome SP1600, Leica, Germany). The sections were polished using silicon carbide paper and a variable speed grinder-polishing machine (Buehler, model Metaserv 2000). These sections were observed microscopically using polarized or normal light. For each sample, a series of 15 µm sections perpendicular to the bone biopsy were cut without decalcification using a microtome (Reichert-Jung Supercut 2050, Vienna, Austria). These thin sections were then stained with Solochrome-cyanine R, Goldner's trichrome and Movat's pentachrome. After mounting on glass slides, the thin sections were observed with light microscopy (Olympus, Tokyo, Japan).

Additionally, the biopsy from case 2 was characterized using transmission electron microscopy (TEM). Sticks with sections of a few mm^2 corresponding to the core of the implant were cut using a diamond saw and embedded in a new resin (EPON). Ultra-thin sections about 60 to 150 nm thick were cut using an ultra microtome (Ultracut E - Riechert-Jung). The sections were deposited on copper grids coated with a collodion film and then stained with uranyl acetate and lead citrate solutions. TEM observations were made using a JEOL electron microscope (JEM 1010) operated at 80 KeV.

Results

In this clinical trial, ICPCS was used for filling alveolar sockets immediately after tooth extraction. This injectable bone substitute consisted of CaP ceramic particles with granulometry of 80-200 µm suspended in a saline solution containing 2% of hydroxylpropylmethyl (HPMC) cellulose. As previously studied, the material is injectable without phase separation for a ceramic to solution ratio of around 58/42. ICPCS is a BCP ceramic suspension having appropriate rheological properties for being injectable [19]. It allows filling of complex shaped bone cavities without setting. After suturing and during the entire post-operative period (6 months), ICPCS was not observed leaking outside none of the 18 filled-alveolar sockets. The results for the 11 patients were excellent, with no high degree of pain or signs of inflammation in any of them. Neither adverse clinical reaction nor infectious complications occurred in the post-operative phase in any of the patients. The pain was always level 1 except for one patient who assessed pain at level 3 because an alveolectomy was needed in order to remove the tooth. Two weeks after surgery, sutures were removed and no signs of gingival inflammation were observed in any of the patients.

As illustrated in Figure 1, no radiolucent line between ICPCS and host bone was observed in any of the X-ray images after 6 months of implantation. The absence of radiolucent line

indicated good integration of the biomaterial into the bone with no signs of osteolysis. The surface of the filled defects appeared to be in continuity with the host alveolar bone crest suggesting its adaptation to the level of the implanted site. In some cases, a radiolucent line was visible between the biomaterial and host bone, at the beginning, but its thickness decreased with time (Fig 1, *). Fifteen days after surgery, five out of eighteen roots exhibited a radiolucent line which evolved into a radio-opaque line over time. Six months after surgery, only one the filled sockets still presented a radiolucent line, but was limited to 10% of its contour. In other cases, a radio-opaque line was noticed in the X-rays of the surgical sites. Fifteen days after surgery, 9 roots out of 18 exhibited a radio-opaque line. After 6 months, 7 roots still showed this radio-opaque line. As shown in Figure 2, the radiographic density of the ICPCS-filled defect tented toward that of the host bone with increase in post-surgery time. Nevertheless, the implanted sites were still visible after 6 months in all cases. The radiographic density of the ICPCS-filled defects was not significantly different between day 0 and day 15 ($p>0.99$), or between day 90 and day 180 ($p=0.128$). However, it was statistically different between 15 days and 3 months after implantation ($p=0.016$) suggesting that bone growth occurred during this period.

A summary of the above-mentioned results on radiolucency and radiographic density is given in Table 1.

Three years after surgery, three patients gave formal consent for a small biopsy of the surgical site to be taken in order to study the *in vivo* behavior of the ICPCS. As shown in Figure 3, X-ray images revealed a significant disappearance of the implantation site after 3 years. A residual implantation image, about 20 % of the initial defect, was still visible in cases 1 and 2. For case 3, the X-ray bone structure was normal with no image of the implanted biomaterial. Clinical observations of the surgical sites indicated normal physiological tissue for the 3 patients. A small incision of the gum revealed the presence of bone on the top of the crest

with ceramic granules still visible in soft tissue for cases 1 and 2 (Figure 4). For case 3, the entire surface was made of hard bone.

In these three patients, a small biopsy of the residual soft and hard tissue was taken and analyzed. Figure 5 shows the SEM micrographs and histology pictures of the biopsy taken under the gingival surface in case 1. Neither the SEM nor the histological sections of this biopsy revealed mineralized bone between the ceramic particles in the upper gingival area. Mineralized bone was only present in the deepest region of the biopsy (Fig. 5a, *). In the central area of the sample, histological sections showed normal fibrous tissue with numerous fibroblastic cells, blood vessels and a small quantity of osteoid tissue between the particles (Fig. 5b,c and d). The SEM micrographs, µCT and histology images of the biopsy taken from case 2 are shown in Figure 6. In this case, mineralized bone was observed between the BCP particles in the lowest region of the biopsy, as evidenced by SEM images (Figure 6a). At high magnification, lamellar bone structure with osteocyte lacunae appeared in direct contact with the ceramic particles, corroborating the bioactivity and osteconductivity of the BCP (Figure 6b). A significant quantity of mineralized bone tissue with 3 dimensional interconnections was observed between the BCP particles in the µCT image (Fig. 6c). Histological sections indicated normal physiological fibrous tissue, osteoid tissue and abundant mineralized bone (Figure 6d). TEM micrographs of this biopsy are shown in Figure 7. In the upper soft tissue region of the biopsy, micro crystals of BCP granules appeared to be encapsulated by collagen fibres and surrounded by elongated cells (Figures 7a,b and c). TEM micrographs of the lowest region of the biopsy revealed mineralized and orientated collagen fibres in direct contact with the BCP micro crystals (Figure 7d). The collagen fibers can be observed during mineralization (Figure 7e). In the center of the BCP granule, there is no collagen fiber visible but biological crystal precipitation was clearly observed between the micro crystals (Figure

7f). The micro crystals were much larger in the center than at the edge of the BCP granules where the mineralization of collagen fibers was observed.

For case 3, the radiographic density of the ICPCS -filled defect was similar to that of host bone and the contour of the defect could not be distinguished after 3 years. After opening the gingival tissue, the bone seemed clinically physiological without soft tissue. The biopsy was removed with force due to the hardness of the bone. Figure 8 illustrates the biopsy taken from case 3. SEM micrographs show abundant mineralized bone formed between some residual BCP granules (Fig 8a). Mineralized bone revealed osteocyte lacunae with a lamellar structure and some regions resembling woven bone. The µCT image (Fig 8b) shows a significant quantity of bone ingrowth around the BCP granules with 3 dimensional interconnections. After removing mineralized bone from two µCT reconstructions, only a few BCP granules were found, suggesting a more active resorption process of the ceramic in this patient than in the others. Polarized light microscopy corroborated the previous observations with oriented collagen fibers (Fig 8c). The histological staining revealed a higher amount of bone ingrowth with little osteoid tissue (red) and a lower amount of BCP granules than in the other 2 patients.

Discussion

We have shown for the first time that an ICPCS can be used to fill dental sockets after tooth extraction with no accompanying adverse effects in humans. ICPCS was composed of 80-200 µm BCP ceramic particles suspended in a viscous solution of cellulose derivative polymer. The clinical outcomes were excellent with no signs of infection, inflammation or osteolysis. The BCP granules supported bone growth and preserved the height of the alveolar bone crest. Preservation of the alveolar bone crest is very important for subsequent dental implantology [24] and prosthetic restoration [25].

Injectable materials composed of resorbable polymers and bioactive granules have been proposed by many authors [26,27,28]. For instance, particulate bioactive glass S53P4 and Poly(ε-caprolactone-co-D,L-lactide) as a thermoplastic carrier matrix were investigated as bone fillers in cancellous and cartilagineous subchondral bone defects in rabbits [29]. This composite was injectable at 47-50°C and set in the bone defect at body temperature. However, the percentage of new bone ingrowth into this composite was low after 6 months of implantation, approximately 6-8%. This injectable material was too dense in relative terms, with very low resorption kinetics for allowing clinically relevant bone growth.

As an alternative, calcium phosphate cements (CPCs) are challenging materials for filling bone defects. In a previous study [30] we showed that CPC injected into a critical sized femoral defect in rabbits provided with good osteoconductivity on its surface after 3 weeks. However, the CPC was not macro porous and thus, acted as a barrier for bone ingrowth toward the center of the defect. Furthermore, the resorption process of the material was minimal even within tiny peripheral fissures which were accessible for cellular activity. This limited degradation hampered the bone substitution process. In the same model, the whole implant area filled with the ICPCS was uniformly colonized by newly-formed bone. These results indicated that BCP particles supported bone formation and that the cellulose polymer (2-3 % w/w) in solution was not a barrier for bone ingrowth. The suspension of BCP granules could be considered as an interconnected macroporous ceramic, as evidenced by 3D reconstruction using the synchrotron facility [30,31]. The intergranular spaces provided with a total open porosity rapidly invaded by body fluids, cells and tissues. Other studies using CPC have reported poor bone growth in various animal models and even in clinical evaluation [32,33,34]. The cement composed of carbonated apatite (NORIAN SRS) was used as a bone filler for different applications in maxillofacial or in orthopaedic surgery. In the study by Wolff *et coll*., the material seemed radiologically to be completely replaced by bone tissue

after 30 months. However, the X-ray image showed a higher density in the filled mandibular region than that of host bone 36 months postoperative. Light microscopy and TEM images of a biopsy taken from the implantation site 6 months after surgery showed bone and fibrous tissue around, but not inside, the cement filler. These results show that biopsy of the implantation site is highly valuable for understanding the biological behaviour of IBSs. In a study with 14 patients [34], the same cement was used to fill displaced tibia plateau fractures. After an average postoperative period of 28 months, the X-ray evaluation of cement resorption was scored as absent, discrete, or substantial. In contrast, the present results show a significant decrease in the visible surface of the implantation site for two patients and total disappearance for one patient after 3 years. In our case, the injectable bone substitute material possesses fully interconnected porosity that allows bone ingrowth between the BCP particles. SEM and TEM observations of the biopsies corroborated a direct apposition of bone tissue due to the precipitation of biological apatite between the micro crystals.

The present clinical evaluation revealed that ICPS is a valuable material for filling dental sockets after tooth extraction because it preserved the alveolar bone crest, supported bone healing and was gradually substituted by bone tissue. The increasing number of partially or completely edentulous patients is closely related to the ageing of the population. More comfortable and durable prosthetic rehabilitation is a great challenge for the scientific and dental community. The esthetic and functional requirements of patients are increasingly important and cannot be satisfied without sufficient bone volume [35]. Various bone fillers including HA, ß-TCP or BCP ceramics have been used to prevent the resorption of the alveolar bone crest or in sinus lift. When used for alveolar filling, the best resorption/substitution outcome was reported with granular formulation rather than with blocks. However, the difficulty of handling particles into exiguous tooth sockets discouraged many dental surgeons. We have recently proposed associating BCP granules with a polymer

solution to provide a ready-to-use IBS [17, 19, 30, 36,37]. The osteoconductive potential of this innovative biomaterial has already been demonstrated using different animal models in our laboratory with the quantification of each component (BCP, minerlaized bone and soft tissue) [18,38]. Other products such as nanocrystalline precipitated hydroxyapatite Ostim (Heraeus Kulzer, Hanau, Germany) have been proposed for alveolar filling. This material contains about 40% of nanometer-scale calcium phosphate apatite particles dispersed in water, has a viscous, fluid-like consistency and can therefore be directly injected into a defect. Despite that HA is normally considered as non-resorbable, a complete resorption of the material was observed at 12 weeks using an adult domestic pig model [39]. It is considered that the resorption process depends mainly on the chemical composition and particle size, both could affect dissolution in body fluids and cellular activity (macrophages, osteoclasts). In our clinical study, residual BCP granules of 80-200 µm were still present but had greatly diminished in size and quantity after 3 years. In a pre-clinical dog model, we have shown that ICPCS composed of 40-80 µm BCP granules disappeared completely 3 month after implantation into freshly extracted dental sockets [24]. In the same animal model and after the same implantation period, the majority of 200-500 µm granules were still present [38]. Similar observations have also been made in critical sized femoral defects in rabbits [18]. Malard *et al*. [40] described a high inflammation process with TRAP-positive cells together with a high bone growth rate using biphasic calcium phosphate particles with size inferior to 10 µm implanted in femoral defects of rats. All of these studies indicated that the size of the particles in the bone filler should be adapted to the clinical indications. For pre-prosthetic surgery, large granule size with slow degradation rate should be preferably used in order to maintain sufficient bone levels. For pre-implantation surgery, small granules compatible with acceptable levels of inflammation should be favoured because they should be rapidly substituted by mineralized bone in dental sockets and will hinder subsequent implantology.

Another way to increase the bioactivity is to coat a P-15 peptide on to the surface of calcium phosphate particles which are then suspended into a hyaluronate hydrogel to form an injectable bone filler [41]. It has been shown that the peptide increased the adhesion of osteoblastic cells onto the ceramic particles.

A review of sinus floor augmentation [42] revealed that interpretation of the results of most clinical studies is hampered by factors such as lack of quantitative or histomorphometric data, differences in follow-up periods, implantation sites, autogenous bone to ceramic mixture ratios, and the use of barrier membranes. In this meta-analysis, the calcium phosphate ceramics were either bovine bone mineral (BBM), HA or β-TCP ceramic particles. HA alone showed more bone volume (18.7%) than BBM alone (8.0%) or BBM mixed with venous blood (14.7%) while β-TCP had the highest amount of bone after 6 months of healing (29.7%). Despite the differences between the materials used, composites of autogenous bone with HA or BBM appeared to produce almost the same amount of bone volume after 6 months. Although BBM is often regarded as a resorbable material, data from clinical histology indicated that BBM particles have not been resorbed and replaced by mineralized bone over time. On the other hand, pure-phase β-TCP resorbed completely and led to about 30% of bone after 6 months. Gaasbeek *et al*. reported the same results [43] with β-TCP in open wedge osteotomies. In these studies, a radiological classification system was used to monitor bone healing with resorbable substitutes [43,44]. This radiological classification system has been correlated with histological findings. Both radiology and histology corroborated almost a complete resorption of the β-TCP material and its replacement by mineralized bone [43,44]. The present results showed a similar correlation between radiographs and histology of BCP ceramic granules implanted in alveolar dental sockets. Another study in human sinus floor augmentation using β-TCP [45] showed, after 6 months of healing, that the borders of the round particles of TCP were partly replaced by bone and

infiltrated by soft connective tissue. Another clinical report corroborated these histological observations on tissue healing within bone filler [46]. Severe periodontal lesions with a one-walled intrabony defect were filled with BBM particles and covered by a bovine peritoneum-derived collagen membrane. Eight months after surgery, a bone-like tissue replaced the host tissues around the BBM near the residual bony wall. Nevertheless, the BBM particles were surrounded by soft connective tissue without osteoblasts in the area far from the bone-wall. In the present study, similar findings were observed with a mixture of mineralized bone in contact with the BCP granules and soft connective tissue just under the gum for 2 patients. In the apical area, bone healing was more favourable due to the proximity of the alveolar host bone. For the third patient, histology revealed that the entire implantation zone was colonized by mineralized bone grater than soft connective tissue. The previous and present histological observations indicated that a competition exists between these two types of tissues and that it is related to the environment of the surgical site. It is rather difficult to know however which tissue will replace the other over time.

The filling of dental sockets by calcium phosphate particles after tooth extraction proved to be a good means for preventing alveolar bone resorption. The maintenance of a sufficient bone level is crucial for dental implantology but the presence of residual filling material should be considered. Brunel *et al*. [47] showed that the presence of HA particles in the regenerated bone had no influence on the osseointegration of implants, presenting a success rate of 86% after a 7-year observation period. This study confirmed the possibility of regenerating bone by means of osteoconductive and resorbable materials, assuring at the same time the long-term success for implants inserted into regenerated sites. In another study [48], the BBM material, Bio-Oss®, was used for maxillary sinus augmentation prior to the placement of titanium dental implants. The success rate of this clinical procedure was reported to be 89.5%.

Although the resorption of particulate ceramics is slow, it did not seem to hinder the placement and biomechanical stability of dental implants.

Conclusion

In an attempt to prevent bone crest resorption after tooth extraction, the present study investigated the efficiency of an injectable bone substitute (ICPCS) as a safe and osteoconductive substitute after filling freshly extracted dental roots in humans. This material would be particularly interesting in conventional or implant-supported prosthetic rehabilitation and could increase the number of candidates for oral implantology. In this context, our data open new therapeutic windows for pre-implant surgery. However, this hypothesis is to be confirmed by further experimentation over a longer period of time with a larger number of patients.

Acknowledgments

This clinical evaluation was financially supported by Nantes Hospital through the Clinical Research Department and the Central Pharmacy, the Regional contract (CPER Biomateriaux S3, Region Pays de la Loire) and the company Biomatlante SAS. We also thank L. Hamel, A. Jean, C. Ballereau, A. Moreau, O. Gauthier, S. Delplace, F. Moreau, J.-F. Bodic and I. Falconi for their advice and help.

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Table 1: Radiographic assessments of the integration of the ICPCS into host bone after filling alveolar dental sockets. X-rays were taken 15 days, 3 and 6 months after surgery (18 fillings in 11 patients; first clinical evaluation).

Time after surgery (days)	Radio-clear line	Radio-opaque line	Radiographic density (%)
0	5/18	3/18	61 ± 12
15	5/18	5/18	59 ± 13
90	3/18	8/18	76 ± 10
180	1/18	7/18	84 ± 12