The association of hydrogel and biphasic calcium phosphate in the treatment of dehiscence-type peri-implant defects: an experimental study in dogs

Xavier Struillou · Mia Rakic · Zahi Badran · Laure Macquigneau ·
Caroline Colombeix · Paul Pilet · Christian Verner ·
Olivier Gauthier · Pierre Weiss · Assem Soueidan

Abstract Hydrogel polymers have many applications in regenerative medicine. The aim of this study in dogs was to investigate bone regeneration in dehiscence-type peri-implant defects created surgically and treated with (i) biphasic calcium phosphate (BCP) granules alone; (ii) a composite putty hydroxypropyl methylcellulose (HPMC)/ BCP (MBCP/putty); and (iii) a polymer crosslinked membrane of silanized-HPMC (Si-HPMC/BCP) compared with empty controls. At 3 months, new bone formation was significantly more important in defects filled with HPMC/ BCP or Si-HPMC/BCP compared with spontaneous healing in control ($P = 0.032$ and $P = 0.046$ respectively) and more substantial compared with BCP alone. Furthermore, new bone formation in direct contact with the implant surface was observed in all three groups treated with BCP. The addition of HPMC to the BCP granules may have enhanced the initial stability of the material within the blood clot in these large and complex osseous defects. The Si-HPMC hydrogel may also act as an occlusive membrane covering the BCP, which could improve the stability of the granules in the defect area. However, the crosslinking time of the Si-HPMC is too long for easy handling and the mechanical properties remain to be improved. The composite MBCP/putty appears to be a valuable bone-graft material in complex defects in periodontology and implantology. These encouraging results should now be confirmed in clinical studies.

1 Introduction

Dental implant therapy is a widely accepted treatment modality that improves the quality of life of patients and shows high success rates and predictability. However, late failures can occur over time, as a result of peri-implant disease (peri-implantitis) or biomechanical overload. Peri-implantitis has been defined as an inflammatory reaction associated with loss of supporting bone around the dental implant [1]. The prevalence of peri-implantitis ranges between 18–56 % of subjects and 9–40 % of implants [2, 3]. Due to bacterial contamination on the surface of the implant, treatment of peri-implantitis primarily focuses on removing the biofilm and decontaminating the implant surface before subsequent bone regeneration. However, the results of the adjunctive use of a graft material remain unpredictable in terms of bone filling and re-osseointegration. Several different biomaterials, i.e., autogenous bone grafts, bovine bone xenografts or alloplastic bone grafts, have been proposed in the surgical management of peri-implant osseous defects. Frequently, the morphology of peri-implant defects is unfavorable for regenerative procedures with large circular or dehiscence defects, which are associated with loose surrounding bone-walls [4].

X. Struillou · Z. Badran · L. Macquigneau · C. Colombeix ·
P. Pilet · P. Weiss · A. Soueidan (✉)
Laboratory of Osteo-Articular and Dental Tissue Engineering
(LIOAD), INSERM, U791, 1 Place Alexis Ricordeau,
44042 Nantes, France
e-mail: assem.soueidan@univ-nantes.fr

X. Struillou · Z. Badran · L. Macquigneau · C. Verner ·
A. Soueidan
Department of Periodontology, UFR d'odontologie,
Nantes, France

M. Rakic
Department of Periodontology, Faculty of Dental Medicine,
University of Belgrade, Belgrade, Serbia

O. Gauthier
Department of Small Animal Surgery, ONIRIS, College of
Veterinary Medicine, Nantes, France

Various preclinical models have been described, such as dehiscence-type defects [5] and critical size supra-alveolar peri-implant defects [6]. These experimentally created defects do not regenerate spontaneously and thus allow the evaluation of biomaterials that may induce bone regeneration around the implants. Alloplastic biomaterials such as biphasic calcium phosphate (BCP) [7, 8] provide an alternative to allografts or xenografts [9, 10] with no risk of viral transmission [11]. BCP is a combination of hydroxyapatite (HA) and tricalcium phosphate (β-TCP) in a variable weight ratio (60/40 or 80/20) and particle size (mainly 80–200 μm or 0.5–1 mm). The major interest of this type of biomaterials is the combination of a short-term (β-TCP) with a long-term (HA) absorbable phase. BCP has been evaluated in various preclinical and clinical studies in periodontology [12, 13] and implantology [14, 15] and may be considered a predictable osteoconductive scaffold for new bone formation [16, 17]. The stability of the wound and of the blood clot during the first healing phases is essential to optimize bone regeneration and the final clinical outcomes. The use of granules remains problematic for surgeons because of the difficulties in handling and maintaining the material into complex osseous defects. Furthermore, a partial loss of material always occurs during healing, due to the pressure of soft tissues and the mechanical stress of the muscular activity. Various biomaterials, such as polymers, have been proposed as cohesive granule linkers in order to improve the handling during the surgical procedure and the stability of the bone-filling material in the wound. Polymers meet the biological requirements to serve as valuable BCP granule linkers. An injectable composite hydrogel/BCP has been tested for use in orthopedic [18] and dental surgery to prevent alveolar ridge resorption prior to placement of dental implants [19, 20]. When implanted in osseous defects, hydrogel is resorbed during the initial healing process to promote biomaterial granule colonization by osseous cells [21]. Hydroxypropyl methylcellulose (HPMC) serves as an excipient vehicle linking BCP granules. In guided bone regeneration (GBR), absorbable membranes are used to cover the bone filling material in order to enhance stability and prevent the loss of granules outside the wound. However, the use of regenerative membranes presents some limitations due to the significant risk of complications such as infection or early exposure [22]. A self-reticulating absorbable polymer based on silanized HPMC (Si-HPMC) has been developed [23, 24] and shows good biocompatibility. Due to its crosslinking and elastic properties, Si-HPMC may serve as a GBR barrier to enhance the stability of the bone-filling material. The gelation process delays cell and tissue colonization by slowly degrading the Si-HPMC [25] and may prevent the invagination of epithelia and soft tissues between the BCP granules.

In a previous pilot study in dogs, we studied the bone filling and regenerative capacities of injectable Si-HPMC/BCP in periodontal defects, and obtained encouraging results [26].

In this present study, using a larger number of animals and defects, we investigated osteoconduction, new bone formation, and bone-to-implant contact in surgically created peri-implant defects filled with three different materials: (i) BCP; (ii) BCP mixed with HPMC (MBCP putty); and (iii) BCP and Si-HPMC used as a membrane for GBR. We then qualitatively and quantitatively analyzed the osteoconductive properties of the three biomaterials, the biodegradation of the ceramic, the bone-to-implant contact, and the percentage of bone ingrowth, by histology, histomorphometry, and image analysis using scanning electron microscopy (SEM). The study was designed according to the ARRIVE guidelines [27] adapted for preclinical in vivo research in implant dentistry [28–30].

2 Materials and methods

2.1 Study design

Four different groups were evaluated in this preclinical study in dogs:

- A control group in which the surgically created peri-implant osseous defects were kept empty. Spontaneous bone healing was expected in this group.
- A second group in which the surgically created peri-implant osseous defects were filled with BCP granules. This treatment may be considered the reference in the management of peri-implant defects.
- A third group in which the surgically created peri-implant osseous defects were filled with a commercial combination of BCP granules and polymer derived from cellulose.
- A fourth group in which the surgically created peri-implant osseous defects were filled with BCP granules and covered with a self-crosslinking polymer derived from cellulose used as a hydrogel membrane of GBR.

2.2 Biomaterial characterization

2.2.1 BCP granules (SBS 60/40®)

The BCP granules (SBS 60/40®, Biomatlante SARL, Vigneux de Bretagne, France) were particles ranging from 500 μm to 1 mm in diameter, in a weight ratio of 60 % HA and 40 % β-TCP. The BCP granules were packaged in plastic syringes and steam sterilized at 121 °C for 30 min.

2.2.2 Composite hydrogel/BCP material (MBCP Putty®)

The composite material (MBCP Putty®, Biomatlante SARL, Vigneux de Bretagne, France) was composed of BCP granules associated with a cellulosic excipient vehicle of pharmaceutical-grade quality in aqueous solution (HPMC, Colorcon-Dow Chemical, France). The HPMC presented an average molecular weight of 290 g/mol and a concentration of 4.4 %. The BCP granules ranged from 500 μm to 1 mm in diameter, with a weight ratio of 60 % HA and 40 % β-TCP. The weight ratio between the HPMC hydrogel and the BCP granules was 50/50. The composite product was poured into glass bottles and steam sterilized at 121 °C for 30 min.

2.2.3 Hydrogel used as a regenerative membrane (Si-HPMC)

Hydroxypropyl methylcellulose (Colorcon-Dow Chemical, France) presents an average molecular weight of 290 g/mol. Silanol groups were grafted onto the HPMC polymer [31]. The silicon percentage (0.46 wt%) grafted onto HPMC was determined using inductively coupled plasma atomic emission spectroscopy (ICP-AES). The Si-HPMC powder was dissolved in NaOH (0.2 M) with constant stirring for 48 h. The resulting viscous solution of Si-HPMC (4 wt%) was dialyzed in diluted NaOH (0.09 M). The polymer solution had a pH of 12.9. Finally, it was poured into glass bottles and steam sterilized at 121 °C for 30 min [27]. The basic viscous Si-HPMC solution was crosslinked by adding an acidic buffer to build a hydrogel. The acidic-buffered solution (pH 3.6, 100 mL) was prepared using HEPES (3.1 g), NaCl (1.46 g), and HCl (0.1 M, 30 mL) to achieve both final physiological osmotic pressure and pH. The addition of the acidic-buffered solution to the viscous Si-HPMC solution was made extemporaneously during surgery, under aseptic conditions.

2.3 Animals

Dogs are the most commonly used animal model in research on bone regeneration around implants [32]. Animal handling and surgical procedures were conducted according to the guidelines of the European Community for the care and use of laboratory animals (2010/63/UE) and approved by the Animal Welfare Committee of ONIRIS, the College of Veterinary Medicine. Six adult beagle dogs were purchased from a professional stockbreeder (mean age 48 ± 2 months, mean weight 16 ± 1 kg). During the experiments, dogs were fed with a soft food diet, with water available ad libitum, and housed in collective cages.

2.4 Surgical procedure

Animal experiments were performed in two separate surgical stages. Dogs received professional scaling 1 week prior to each surgical stage, under general anesthesia.

Surgeries were performed under general anesthesia using an intravenous injection of diazepam (Valium®, Roche, France) 0.25 mg/kg, propofol (Rapinovet®, Schering Plough, United Kingdom) 4 mg/kg. A single dose of morphine (Morphine, Cooper, France) 0.1 mg/kg was injected subcutaneously during surgery as an analgesic. Local anesthesia with bupivacaïne 0.50 % (Bupivacaïne®, Aguettant, France) was also administered in mandibular blocks. After general anesthesia, the gingiva was disinfected with iodine solution, and the animal was covered with a sterile drape.

During the first surgical stage, the mandibular first and second molars (M1 and M2) were extracted bilaterally in the six dogs. Atraumatic surgical procedures (tooth separation) were used to preserve bone. Wound closure was accomplished using mattress sutures with absorbable sutures (Vicryl® 3.0, Ethicon, France) and the sites were allowed to heal for 3 months. Prophylactic antibacterial treatment was administrated after surgery (Stomorgyl®, spiramycin + Metronidazole, Merial, France). There were no complications during the healing period.

During the second surgical stage, after 3 months of spontaneous bone healing, a mucoperiosteal flap was reflected at the level of the mandibular extraction sites. Conventional implant site preparation was performed under saline irrigation, in accordance with the indications of the manufacturer (Zimmer Dental Incorporated, USA). Two implants sites were created on each side of the mandible of all dogs.

After implant site preparation, dehiscence-type bone defects were created as described in the literature [5, 33]. These surgically created bone defects were standardized using a periodontal probe (PCP12, HU-FRIEDY Co., Chicago, IL, USA). The defect sites preparation was performed with a motor-driven drill (Aesculap, Tuttlingen, Germany) and a fissure carbide bur under intensive saline irrigation. The defects were 4 mm in height from the crestal bone, 2 mm in depth and 4 mm in width. Titanium implants were then placed (Zimmer TSV®, diameter 3.7 mm, length 13 mm). All sites showed good primary stability of the implants. Thereafter, each side of the mandible was randomly chosen to serve as a test or control site. All test sites were filled with one of the tested biomaterials and the control sites were kept empty for spontaneous healing (Fig. 1). The flaps were repositioned in a coronal direction and sutured with absorbable sutures (Vicryl® 3.0, Ethicon, France). All surgeries were performed by the same operator (X.S). A total of 24 defects were available, with 6 defects for each treatment group.
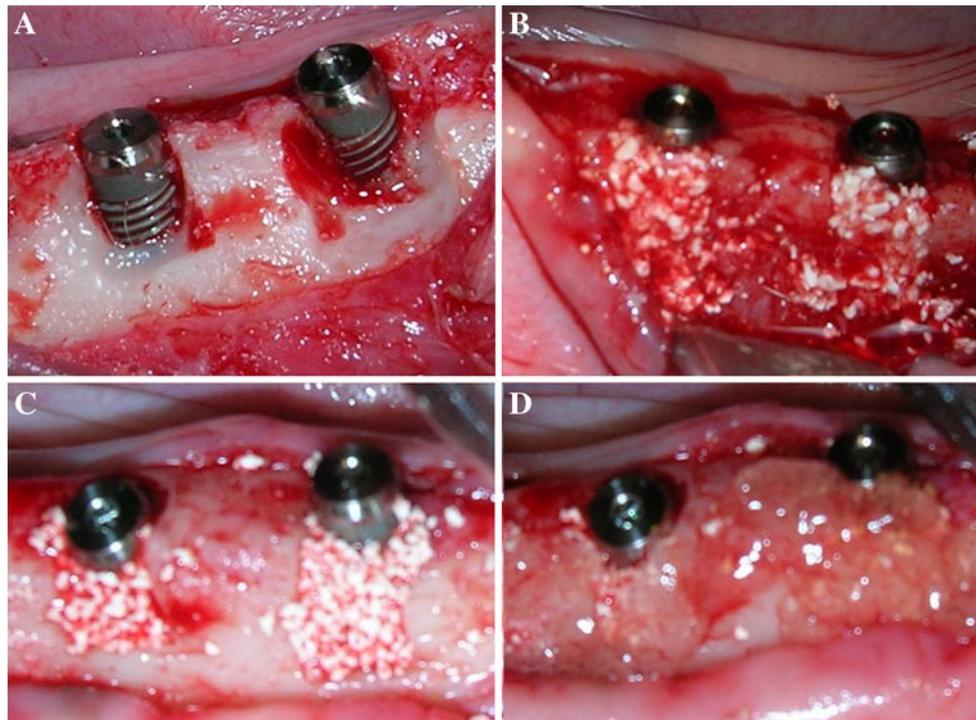
Fig. 1 Dehiscence-type surgically created peri-implant defects. The defects were 4 mm in height from the crestal bone, 2 mm in depth and 4 mm in width. Four conditions were evaluated: **a** empty control with spontaneous bone healing; **b** defects filled with BCP; **c** BCP + HPMC (MBCP putty); or **d** BCP covered by Si-HPMC as a regenerative hydrogel membrane

2.5 Animal sacrifice

After a healing period of 12 weeks, the animals were anesthetized and sacrificed by an intravenous overdose of sodium pentobarbital. Both mandibles were immediately dissected and placed in a formol solution.

2.6 Histological preparation of the samples

The non-decalcified bone samples were dehydrated in an ascending series of ethanol (70–100 %) and then in pure acetone for 24 h. The samples were impregnated in methyl methacrylate (Prolabo) for 4 days and then embedded in a polymethyl methacrylate resin. Blocks were cut into 100-μm think slices with a circular diamond saw (Microtome 1600, Leica, Germany) to preserve the bone/implant interface. The slices were examined by light microscopy after hematoxylin–eosin staining.

2.7 Histomorphometry

For SEM analysis, samples were sputtered with a thin layer of gold–palladium alloy (EM Scope, England). SEM micrographs were taken using the backscattered electron mode at 15 kV (SEM, LEO 1450 VP). Percentages of bone ingrowth as well as bone-to-biomaterial contact were calculated based on backscattered SEM (BSEM) images. Custom-made software with an image analysis system (QWin, Leica, Germany) was used. The BCP particles, bone and non-mineralized tissues were easily discriminated on BSEM images based on their respective grey levels. The area of interest was manually defined by the bottom of the defect apically, the implant lingually and a curve joining the buccal bone to the top of the shoulder of the implant (Fig. 2). The area of the biomaterial, the newly formed bone, and the non-mineralized tissue were calculated (in mm^2). The surface (in mm^2) of the new bone in contact with the implant was also measured. SEM analysis through secondary electrons and backscattered electrons allowed for a qualitative and quantitative investigation of bone formation and ceramic degradation.

Histomorphometry and histology were conducted by the same investigator (L.M.) and validated by a second investigator (X.S.). A calibration procedure was performed prior to analysis.

2.8 Statistical analysis

After manual delimitation of the area of interest, the primary outcome variables were the surface (in mm^2) of
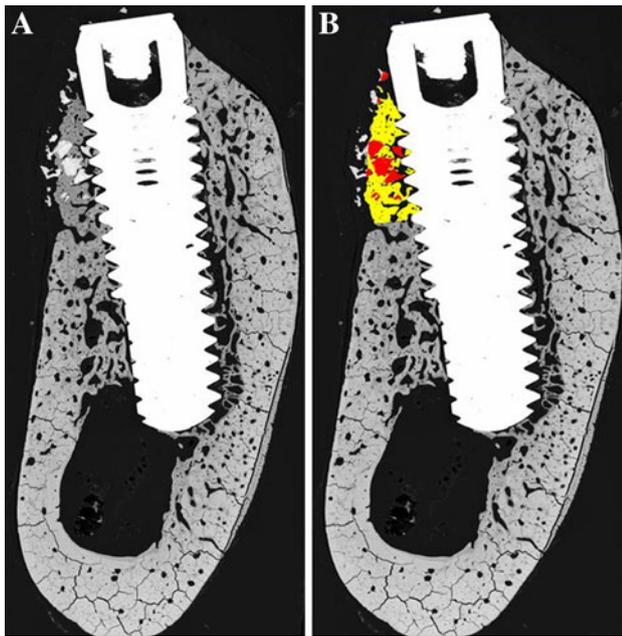
Fig. 2 BSEM pictures of dehiscence-type peri-implant defects filled with BCP + HPMC composite material in putty form. After manual delimitation of the area of interest, newly formed bone appeared in *yellow*, the composite material in *red* and the non-mineralized tissue in *black*. Note that BCP granules that have migrated outside of the defect area into the soft-tissues are not recognized by the software (Color figure online)

mineralized tissue (biomaterial and new bone) and the surface of non-mineralized tissue. Bone ingrowth (expressed as a percentage) was defined as the relative ratio of the surface of new bone and biomaterial to the total surface of the area of interest. All data were expressed as mean and standard deviation (SD) together with median values and 95 % confidence intervals for all variables.

Nonparametric statistical tests were used for analysis because of the relatively small sample size. Intergroup comparisons were made using the Friedman test for repeated measurements, and the Kruskal–Wallis test for relative ratios. Then, the Mann–Whitney test was used to evaluate the differences in measured parameters among groups. In some cases the 'exact' option was performed with the aim to compensate the limitations of sample size. All statistical analyses were performed using SPSS v.18.0 (SPSS Inc., Chicago, IL, USA) and statistical significance was set at 5 % ($P < 0.05$).

3 Results

No complications occurred during the surgeries and post-operative healing was uneventful in all dogs. No infectious complications or premature exposure of the implants were observed throughout the entire study period.

3.1 Histological observations

At 3 months, wound healing in the four groups was characterized by variable new bone formation in the defect area (Fig. 3).

In the control group, the newly formed bone was mainly in direct contact with the existing bone surrounding the defects. No complete healing or regeneration of these peri-implant defects was observed in any of the specimens. In the apical part of the defects, the newly formed bone was in direct contact with the implant. However, the bone regeneration never extended to the most coronal thread of the implant, in any control site. Furthermore, bone regeneration in the buccal direction was quite limited in the medium and upper part of the defects.

In the BCP group, newly formed mineralized bone was observed in direct contact with both the BCP granules and the implant surface. The density of the biomaterial varied depending on the localization in the defect: a higher number of BCP granules was observed at the top of the defect than at the base, where bone formation was more extensive. In this coronal area, BCP granules were surrounded by a smooth osteoid tissue (a non-mineralized matrix) and invagination of the gingival tissues between
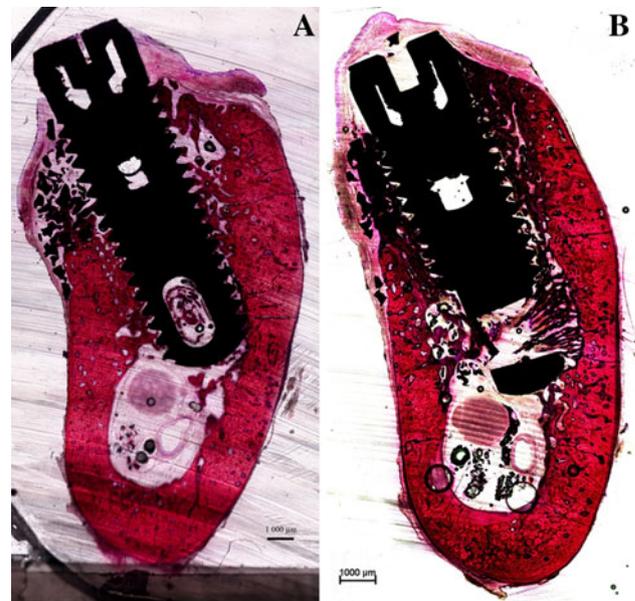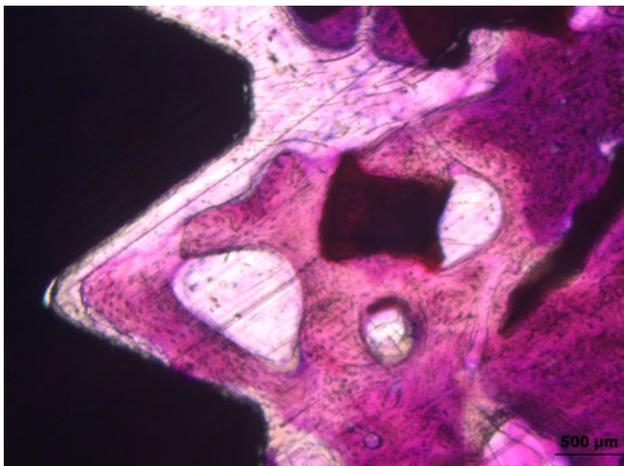

Fig. 3 Histological micrographs of dehiscence-type surgically created peri-implant defects after 3 months (hematoxylin–eosin). Defects filled with **a** BCP and covered by Si-HPMC as a regenerative hydrogel membrane; and **b** BCP + HPMC (MBCP putty). Note the more significant migration of BCP granules into the soft tissues in the defect filled with BCP and covered by Si-HPMC compared with the defect filled with the BCP + HPMC composite material in putty form. The newly formed bone extends to the most coronal part of the defect in the two treatment conditions, with a direct contact to the implant surface. BCP granules are mainly located on the buccal part of the defect

the BCP granules was noticed in some areas. Some BCP granules were also observed within the soft tissues, outside the defect area.

In the composite hydrogel/BCP group, new bone formation appeared to be more extensive in the coronal and buccal directions than in the three other groups (Fig. 3). The newly formed bone was found to be in direct contact with the implant surface and invaded the space between two threads of the implant (Fig. 4). In two defects, almost complete regeneration of the defect was obtained. The woven bone was mainly located in the basal and medium parts of the defects. In the coronal part, osteoid tissue was observed surrounding the BCP granules, but new bone formation was rather limited. The BCP granules and osteoid tissue were surrounded by a non-mineralized matrix. However, the material remained in place in the defect during the healing period, and at 3 months, the biomaterial/new bone covered the entire defect area. When compared to the other groups, the MBCP Putty® group appeared to be distinctively more efficient in supporting the bone filling of the defect in all directions. Only a few BCP granules migrated into the soft tissues outside the defect area.

In the Si-HPMC/BCP group, bone formation occurred less extensively in the coronal direction than in the composite hydrogel/BCP test group. As in the others tested groups, the newly formed bone was observed in direct contact with the implant surface and invaded the spaces between two threads of the implant. The BCP granules were surrounded by osteoid tissue and new bone formation occurred between the bone graft particles. In the basal and medium parts of the defects, new bone formation appeared to be quite similar to that obtained in the BCP test group. In the most

Fig. 4 Histological micrographs of dehiscence-type peri-implant defect filled with the composite material BCP + HPMC in putty form. New bone formation occurs between the threads of the implant in a direct bone-to-implant contact. After 3 months, the BCP granules remain observable, but were never in direct contact with the implant surface

coronal part, BCP particles were surrounded by a non-mineralized matrix, as observed in the composite hydrogel/BCP test group. In the buccal wall, the gingival tissue did not invade the external superficial part of the defect. However, some BCP granules were also observed outside the defect area (Fig. 3).

3.2 Qualitative SEM analysis

Qualitative SEM analysis confirmed the histological results (Fig. 5). Indeed, in the control group, the newly formed bone was mainly located in the apical/central parts of the defect and never extended to the most coronal thread of the implant. The newly formed mineralized bone was observed in direct contact with the BCP granules in the three experimental groups (BCP, BCP + HPMC, BCP + Si-HPMC). This well-mineralized bone had osteocytes developed on or between the granules and showed trabeculae bridging the BCP granules. In the BCP + HPMC or BCP + Si-HPMC groups, reconstruction of the buccal cortical bone was more commonly observed. The central and coronal parts of the defects were more extensively filled with bone than was the case in the control group.

3.3 Quantitative SEM analysis

The bone-to-implant contact was more significant in the MBCP Putty® test group, due to greater coronal extension of the newly formed bone. However, in the two other groups (BCP and BCP + Si-HPMC), the bone-to-implant contact was quite similar to that observed in the control group, with spontaneous healing. The results for the control group and the tested groups were not significantly different (Table 1).

The mean values of bone ingrowth after 3 months of healing in the peri-implant surgically created defects in the lower jaw are presented in Tables 2 and 3. Bone ingrowth was 0.49 ± 0.12 ($n = 6$) in the BCP + Si-HPMC group, 0.52 ± 0.16 ($n = 6$) in the BCP + HPMC group, 0.42 ± 0.19 ($n = 6$) in the reference BCP group, and 0.33 ± 0.14 ($n = 6$) in the control group. A significant increase in bone ingrowth values was observed in the BCP + HPMC group compared with the control group ($P = 0.032$) and also in the BCP + Si-HPMC group compared with the control group ($P = 0.046$). The results were not significantly different in the reference BCP group compared with the control group and between all groups with biomaterial.

4 Discussion

Their high success and survival rates have made dental implants increasingly used by clinicians and patients to
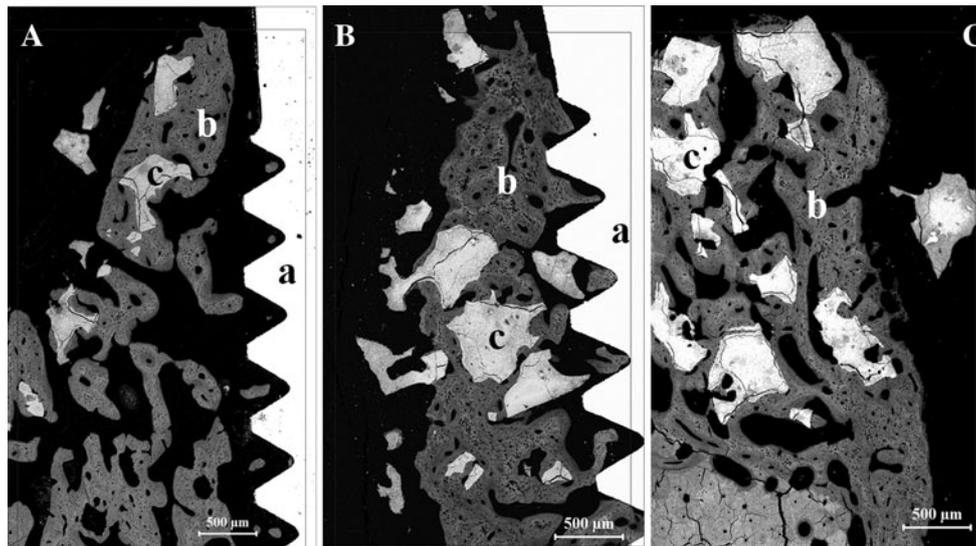
Fig. 5 BSEM pictures of dehiscence-type peri-implant defect filled with **a** BCP; **b** BCP + HPMC; and **c** BCP covered by Si-HPMC. In the three conditions, note the osteoconductive properties of the material: (*a*) implant; (*b*) newly formed bone; (*c*) BCP granules

Table 1 Bone-to-implant contact between the four groups

	BCP + Si-HPMC as hydrogel membrane	BCP + HPMC (MBCP Putty®)	BCP (SBS 60/40®)	Control	
Bone-to-implant contact (in %)	16.08 ± 7.94	20.98 ± 10.45	18.79 ± 13.56	16.34 ± 7.33	1–2: $P = 0.055$**
					1–3: $P = 0.423$**
					1–4: $P = 0.631$**
					2–3: $P = 0.262$**
					2–4: $P = 0.078$**
					3–4: $P = 0.337$**

Values are expressed as mean ± standard deviation

** Non-significant P values ($P > 0.05$)

Table 2 Descriptive statistics of measured parameters of new bone formation in dehiscence-type defects

	1BCP + Si-HPMC as hydrogel membrane	2BCP + HPMC (MBCP Putty®)	3BCP (SBS 60/40®)	Control
Biomaterial surface	0.64 ± 0.59	0.63 ± 0.23	0.76 ± 1.24	0.00 ± 0.00
	(0.47; 0.02–1.26)	(0.71; 0.38–0.87)	(0.35; 0.53–2.05)	(0)
New bone surface	1.53 ± 0.99	2.23 ± 0.97	2.31 ± 1.81	1.72 ± 0.75
	(1.37; 0.49–2.56)	(1.85; 1.20–3.24)	(2.23; 0.40–4.21)	(1.53; 0.92–2.50)
Non-mineralized tissue surface	2.39 ± 1.65	2.73 ± 1.15	3.39 ± 1.56	3.32 ± 0.86
	(1.61; 0.66–4.12)	(2.64; 1.52–3.94)	(2.82; 1.74–5.03)	(3.25; 2.42–4.22)
Bone ingrowth (%)	0.49 ± 0.12	0.52 ± 0.16	0.42 ± 0.19	0.34 ± 0.14
	(0.48; 0.36–0.60)	(0.54; 0.35–0.68)	(0.42; 0.22–0.61)	(0.27; 0.19–0.48)

Values are expressed as mean ± standard deviation and median with confidence interval of 95 %

support fixed or removable prosthodontic rehabilitation. Millions of dental implants are inserted every year worldwide. As in natural dentition, implants are in contact with the bacterial biofilm of the oral cavity and the peri-implant soft tissues form a barrier, which needs to stay healthy for the long-term stability of the implant's rehabilitation. However, inflammatory peri-implant diseases can occur over years and be limited to the peri-implant mucosa (i.e., peri-implant mucositis) or involving the bone support of the implant. Peri-implantitis is defined as the presence of

Table 3 Ratio of bone ingrowth in the four groups

	BCP + Si-HPMC as hydrogel membrane	BCP + HPMC (MBCP Putty®)	BCP (SBS 60/40®)	Control	
Bone ingrowth (ratio)	0.49 ± 0.12	0.52 ± 0.16	0.42 ± 0.19	0.34 ± 0.14	1–2: P = 0.350
					1–3: P = 0.242
					1–4: P = 0.046**
					2–3: P = 0.154
					2–4: P = 0.032**
					3–4: P = 0.196

Values are expressed as mean ± standard deviation

** P value <0.05

inflamed mucosa and bleeding on probing (BOP), pocket depth (PD) of more than 5 mm, cumulative bone loss of more than 2 mm and/or more than three implant threads [2, 34]. The treatment of peri-implantitis remains a real challenge for clinicians. As in periodontal disease, the first goal is to control bacterial infection. Around implants, this means decontaminating the implant surface exposed to the oral biofilm. Non-surgical mechanical cleaning, with or without adjunctive antibacterial treatment, is ineffective as treatment for peri-implantitis [35]. A surgical approach with open flap debridement and systematic antimicrobials appears to be the treatment of choice [36]. Various methods of decontaminating the implant surface have been suggested, such as local antimicrobial therapy, air powder flow and lasers [37]. In order to achieve complete and long-term resolution of peri-implantitis, regeneration of the bone lost during peri-implant disease and re-osseointegration is thought in conjunction with strict maintenance care [3, 36, 37].

Various bone-filling materials have been proposed for the management of osseous peri-implant defects, including autogenous bone grafts, allogeneic decalcified freeze-dried bone, xenogeneic bone mineral, calcium carbonate, HA, tri-calcium phosphate and BCP [38, 39]. The additional use of non-absorbable [(polytetrafluoroethylene (PTFE)] or absorbable (polylactic/polyglycolic) membranes during the healing phase has been suggested to cover and maintain the bone-filling material around implants [39, 40]. As in periodontal intraosseous defects, the morphology of osseous peri-implant defects appears to be a crucial element of successful treatment. Large or dehiscent defects show worse clinical results than circular defects with intact surrounding bone walls [4].

Most bone-filling materials available in dentistry are packaged as granules with various particle sizes. In the last years, new composite materials have been introduced. Their composition is a classical bone filling material (i.e., bovine xenograft or silicate) covered with a layer (i.e., collagen or polyethylene glycol). The layer is added to make the material easier to handle during surgery, to

promote bone regeneration, and to enhance the initial stability of the material into the bone defect during the healing [41]. New materials have also been proposed to serve as membranes in GBR procedures. Thus, chitosan, a deacetylated derivative of chitin, has been studied in various preclinical models [42–44] and may be coated with HA or alginate to enhance the mechanical strength of the membrane [42, 45, 46]. The placement and adaptation of the membrane is often difficult during the surgical phase and complications (especially premature exposure) often occur, which can dramatically reduce bone regeneration. A novel approach with the use of crosslinking gel (Membragel®, Institut Straumann AG, Basel, Switzerland) has been suggested in order to facilitate handling during surgery and to reduce the impact of complications during healing [47].

The aim of the present study was to evaluate new bone formation, in dehiscence-type peri-implant defects filled with BCP granules or with the adjunction of two different derivatives of HPMC (HPMC used as a layer of BCP granules or Si-HPMC used as an occlusive membrane injected over the graft material). The buccal dehiscence type peri-implant defect is a well-documented animal model exhibiting partial spontaneous bone regeneration and is thus a valuable model for the evaluation of new bone-filling materials used in the treatment of peri-implantitis or for GBR [4, 39]. Furthermore, the dehiscence-type defect exhibits clinical conditions similar to those found around oral implants in humans with peri-implantitis. In these open defects, with the loss of the buccal osseous wall, the retention of the bone-filling material and the stability of the clot are unpredictable. This is an appropriate preclinical model to evaluate new bone-filling materials designed to enhance surgical handling, initial stability and bone regeneration.

In the control group, the spontaneous bone regeneration mainly occurred in the basal and medium part of the defects and soft tissues collapsed into the wound area in the coronal and buccal part of the defects. In the group where defects were filled with BCP granules, new bone formation was more significant in the buccal part of the defects,

compared with the control. In the coronal part, new bone formation never reached the implant shoulder. BCP exhibited osteoconductive properties with new bone formed on or between the granules. Furthermore, the newly formed bone appeared to be in direct contact with the threads of the implant with osseointegration in the defect area. Thus, BCP granules may be a valuable bone graft material around implants in the treatment of peri-implant osseous defects or in GBR.

However, in the present study, bone ingrowth with BCP was better than in the negative control group (0.42 ± 0.19 and 0.34 ± 0.14, respectively), although the difference was not statistically significant. These results may be due to the instability of the material during the healing phase in these complex, open osseous defects. In the viscous liquid phase/ BCP test group (MBCP Putty®), the composite material was very easy to handle during the surgical phase, and showed good stability in the defect. After 3 months of healing, we rarely found BCP granules dispersed out of the osseous site. Furthermore, new bone formation appeared to be more extensive in the coronal and buccal parts of the defects, compared with the three other groups. Bone ingrowth was better compared with the control group (0.52 ± 0.16 and 0.34 ± 0.14 respectively) and the difference was statistically significant. Compared with the two other groups with BCP, new bone formation was more significant in the hydrogel/BCP test group, but the difference was not statistically significant (MBCP Putty®/SBS 60/40®: $P = 0.150$; MBCP Putty®/SBS 60/40® + Si-HPMC: $P = 0.350$). These differences in the extent of new bone formation may be due on one hand to the better stability and handling of the material during the surgery and the first healing phase, and on the other hand to the properties of this composite material. In fact, the bone healing process may be favored by the washing of the polymer carrier with biological fluids, leading to the formation of interconnections between the BCP granules. Thus, the angiogenesis and cell colonization of the wound area may occur more quickly [48–50], which is essential for new bone formation and the process of healing [51]. In periodontal or peri-implant osseous defects, the stability of the blood clot is an essential factor in bone and periodontal regeneration [52]. This new composite material was much easier to place into these complex dehiscence type defects, compared with the BCP granules that are seen as the conventional material used for GBR. The initial stability at the end of surgery was clinically improved and the use of a membrane to cover the bone grafting material did not seem necessary. Even if the results of the bone ingrowth were not statistically different between the MBCP Putty® and the SBS 60/40® groups, new bone formation was substantially greater with the composite material. In the two groups, the surgical conditions and bone defects were similar, and the size and structure of the BCP granules were exactly the same. The better results obtained with the composite material may be attributed to the addition of the polymer carrier which enhanced initial stability without compromising the osteoconductive properties of the BCP granules. Previous preclinical and clinical studies have investigated bone regeneration with an injectable composite viscous liquid phase/BCP bone substitute used in dentistry for post-extraction ridge preservation or bone filling of dental sockets, with encouraging results [19, 20]. However, in these studies, the osseous defects showed four bone walls, which is the most favorable clinical situation, and is very far from the morphology of the dehiscence-type defects created here. The polymer carrier was also HPMC but, to be injectable, the BCP granules had to be smaller than those used in the present study (80–200 μm and 0.5–1 mm respectively). This viscous liquid phase did not seem adequate in open and complex osseous defects because of the loss of hardening and lesser initial stability. In a previous study, we used small BCP particles in periodontal complex furcation defects [26] and the material was unable to stay in place in the wound area even during the surgical stage. A putty form, with larger BCP granules, seemed more adapted to the specificities of peri-implant complex defects than the injectable gel form. MBCP putty® does not have initial mechanical properties like hydraulic bone cements [25, 48], but the rapid disappearance [25] of the polymer carrier released space for bone cell migration. In the test group where BCP + Si-HPMC was used as a regenerative hydrogel membrane (SBS 60/40® + Si-HPMC), bone ingrowth was better than in the negative control group (0.49 ± 0.12 and 0.34 ± 0.14, respectively) and the difference was statistically significant. Compared with the BCP group (SBS 60/40®), the new bone formation was more extensive, but the difference was not statistically significant. Given the results of our previous work on furcation defects [26], we increased the viscosity of the Si-HPMC viscous liquid phase from 3 to 4 %. Thus, the crosslinking polymer was easier to handle during the surgical phase. The Si-HPMC acts as an occlusive membrane after gelation [26], but the mechanical properties remain insufficient. Indeed, the polymer membrane fails to prevent the partial leakage of BCP granules outside the defects. After 3 months of healing, more particles could be found in the gingival tissues in the BCP/Si-HPMC group, compared with the MBCP putty® form group. This means that the polymer used as a membrane does not have the ability to resist mechanical forces during healing. However, the improved new bone formation, compared with the use of BCP granules alone (0.49 ± 0.12 and 0.42 ± 0.19, respectively), indicates that the use of a membrane enhances bone regeneration. These results are in agreement with the clinical results available in the literature [4, 39]. The healing was totally uneventful, without premature exposure of the BCP granules,

suggesting that Si-HPMC may be used as a valuable regenerative membrane to cover a bone-graft material. Even if this type of polymer shows good biocompatibility and soft tissue tolerance, the mechanical properties after crosslinking remain to be improved.

The morphology of the defects surgically created in the present study represents one of the most difficult challenges for clinicians in implantology [4]. The loss of buccal osseous wall dramatically restricts spontaneous bone regeneration. Thus, the use of bone-graft material (whether associated with an absorbable membrane or not) is often necessary, with unpredictable results in wide and open defects [57]. Frequently, difficulty in shaping granules correctly compromises the stability of the blood clot and limits new bone formation. Furthermore, the use of a membrane to cover the granules, may lead to premature exposure and a worse result. New generations of bone-filling materials are trying to respond to these clinical challenges in order to make the outcomes of surgical therapy more predictable [21]. In the present study, BCP granules showed high osteoconductive properties both alone and associated with a carrier polymer. They enhanced bone regeneration and enabled new bone formation in direct contact with the threads of the dental implants. Therefore, BCP may be considered as a valuable biomaterial for GBR. The addition of a HPMC polymer carrier enhances the initial stability of the bone-filling material in complex defects. Due to the rapid washing of the excipient vehicle by the biological fluids, new bone formation can occur between the BCP granules [25]. After 3 months of healing, bone ingrowth was better than with BCP alone, especially in the most complex parts of the defects (coronal and buccal). Composite HPMC/BCP material (MBCP Putty®) may be considered as a valuable biomaterial for GBR, especially in complex osseous defects, due to its capacity to enhance the stability of the wound during healing. Furthermore, this material is very easy to handle during surgery and no complications were noticed during the healing phase. The larger BCP granules (0.5–1 mm) in the putty form seem more adapted to use in open and complex defects than 80–200 μm BCP granules of the injectable form previously investigated [19, 20, 53]. However, these encouraging results need to be corroborated by clinical trials in humans. In the future, the polymer may also act as a carrier of stem cells, biologically active molecules, or antimicrobials [54–56]. This approach may be of great interest in the treatment of peri-implantitis, where decontamination of the exposed part of the implant remains unresolved. In dehiscence-type or circumferential peri-implant defects, the use of a bone-graft material enhances new bone formation and the extent of bone/implant contact [57]. However, in these complex and open defects, the bone-graft has to be covered by a membrane for initial stabilization of the granules [39, 40]. The use of polymers as absorbable membranes for GBR has been suggested [58]. Si-HPMC, when used as a hydrogel membrane covering the bone-graft material, enhances new bone formation compared with the use of the BCP granules alone. Due to the barrier effect of Si-HPMC, only limited invagination of soft tissues into the wound was observed. However, the mechanical properties of this polymer hydrogel must be increased further before any clinical evaluation can be performed in humans.

5 Conclusions

The aim of this study was to evaluate bone regeneration in dehiscence-type complex peri-implant defects. For the filling of osseous surgically created defects, three biomaterials were investigated: BCP alone, a composite HPMC/BCP material in a putty form and BCP covered by a polymer barrier of Si-HPMC. These last two materials had never been evaluated around dental implants, even if previous investigations had demonstrated their interest in orthopedic surgery. In peri-implant defects, these three BCP materials showed osteoconductive properties and re-osseointegration of the implants in the defect area, with a newly formed bone in direct contact with the implant surface. New bone formation was enhanced in all groups with biomaterials compared with the spontaneous healing observed in the negative control group. The difference was statistically significant in the putty HPMC/BCP group and in the calcium phosphate/Si-HPMC group. We demonstrated the ability of these composite liquid viscous phase/BCP materials to promote new bone formation, 3 months after implantation, in dog dehiscence-type peri-implant defects. In these unfavorable defects, the putty material provided the best results and was very easy to shape during surgery. Due to the lack of adverse effects during the healing and the encouraging results regarding new bone formation, the putty material can be considered as a valuable scaffold to treat peri-implant defects. However, clinical trials in humans are needed to validate the results of the present study. The viscosity and mechanical properties of the hydrogel remain to be improved before clinical testing in humans.

Conflict of interest The authors have no conflict of interests to declare.

References

1. Albrektsson T, Isidor F. Consensus report of session IV. In: Lang NP, Karring T, eds. Proceedings of the 1st European Workshop on Periodontology. Quintescence, Berlin 1994:365–369.

2. Klinge B, Meyle J, Working Group 2. Peri-implant tissue destruction. The Third EAO Consensus Conference 2012. Clin Oral Implants Res. 2012;23(6 Suppl):108–10. doi:10.1111/j.1600-0501.2012.02555.x.

3. Atieh MA, Alsabeeha NH, Faggion CM Jr, Duncan WJ. The frequency of peri-implant diseases: a systematic review and meta-analysis. J Periodontol. 2012;13. [Epub ahead of print].

4. Schwarz F, Sahm N, Schwarz K, Becker J. Impact of defect configuration on the clinical outcome following surgical regenerative therapy of peri-implantitis. J Clin Periodontol. 2010;37(5):449–55. doi:10.1111/j.1600-051X.2010.01540.x.

5. Schwarz F, Sager M, Ferrari D, Herten M, Wieland M, Becker J. Bone regeneration in dehiscence-type defects at non-submerged and submerged chemically modified (SLActive) and conventional SLA titanium implants: an immunohistochemical study in dogs. J Clin Periodontol. 2008;35(1):64–75.

6. Wikesjö UM, Susin C, Qahash M, Polimeni G, Leknes KN, Shanaman RH, Prasad HS, Rohrer MD, Hall J. The critical-size supraalveolar peri-implant defect model: characteristics and use. J Clin Periodontol. 2006;33(11):846–54.

7. Pandit N, Gupta R, Gupta S. A comparative evaluation of biphasic calcium phosphate material and bioglass in the treatment of periodontal osseous defects: a clinical and radiological study. J Contemp Dent Pract. 2010;11(2):025–32.

8. LeGeros RZ. Calcium phosphate-based osteoinductive materials. Chem Rev. 2008;108(11):4742–53. doi:10.1021/cr800427g.

9. AlGhamdi AS, Shibly O, Ciancio SG. Osseous grafting part II: xenografts and alloplasts for periodontal regeneration—a literature review. J Int Acad Periodontol. 2010;12(2):39–44.

10. AlGhamdi AS, Shibly O, Ciancio SG. Osseous grafting part I: autografts and allografts for periodontal regeneration—a literature review. J Int Acad Periodontol. 2010;12(2):34–8.

11. Daculsi G, Laboux O, Malard O, Weiss P. Current state of the art of biphasic calcium phosphate bioceramics. J Mater Sci Mater Med. 2003;14(3):195–200.

12. Jensen SS, Bornstein MM, Dard M, Bosshardt DD, Buser D. Comparative study of biphasic calcium phosphates with different HA/TCP ratios in mandibular bone defects. A long-term histomorphometric study in minipigs. J Biomed Mater Res B. 2009;90(1):171–81.

13. Peres MF, Ribeiro ED, Casarin RC, Ruiz KG, Junior FH, Sallum EA, Casati MZ. Hydroxyapatite/β-tricalcium phosphate and enamel matrix derivative for treatment of proximal class II furcation defects: a randomized clinical trial. J Clin Periodontol. 2013;40(3):252–9. doi:10.1111/jcpe.12054.

14. Froum SJ, Wallace SS, Cho SC, Elian N, Tarnow DP. Histomorphometric comparison of a biphasic bone ceramic to anorganic bovine bone for sinus augmentation: 6- to 8-month postsurgical assessment of vital bone formation. A pilot study. Int J Periodontics Restor Dent. 2008;28(3):273–81.

15. Cordaro L, Bosshardt DD, Palattella P, Rao W, Serino G, Chiapasco M. Maxillary sinus grafting with Bio-Oss or Straumann bone ceramic: histomorphometric results from a randomized controlled multicenter clinical trial. Clin Oral Implants Res. 2008;19(8):796–803. doi:10.1111/j.1600-0501.2008.01565.x.

16. Friedmann A, Dard M, Kleber BM, Bernimoulin JP, Bosshardt DD. Ridge augmentation and maxillary sinus grafting with a biphasic calcium phosphate: histologic and histomorphometric observations. Clin Oral Implants Res. 2009;20(7):708–14. doi:10.1111/j.1600-0501.2009.01708.x.

17. Bodde EW, Wolke JG, Kowalski RS, Jansen JA. Bone regeneration of porous beta-tricalcium phosphate (Conduit TCP) and of biphasic calcium phosphate ceramic (Biosel) in trabecular defects in sheep. J Biomed Mater Res A. 2007;82(3):711–22. doi:10.1002/jbm.a.30990.

18. Boix D, Gauthier O, Guicheux J, Pilet P, Weiss P, Grimandi G, Daculsi G. Alveolar bone regeneration for immediate implant placement using an injectable bone substitute: an experimental study in dogs. J Periodontol. 2004;75(5):663–71.

19. Boix D, Weiss P, Gauthier O, Guicheux J, Bouler JM, Pilet P, et al. Injectable bone substitute to preserve alveolar ridge resorption after tooth extraction: a study in dog. J Mater Sci Mater Med. 2006;17(11):1145–52. doi:10.1007/s10856-006-0542-7.

20. Weiss P, Layrolle P, Clergeau LP, Enckel B, Pilet P, Amouriq Y, et al. The safety and efficacy of an injectable bone substitute in dental sockets demonstrated in a human clinical trial. Biomaterials. 2007;28(22):3295–305. doi:10.1016/j.biomaterials.2007.04.006.

21. Daculsi G, Uzel AP, Weiss P, Goyenvalle E, Aguado E. Developments in injectable multiphasic biomaterials. The performance of microporous biphasic calcium phosphate granules and hydrogels. J Mater Sci Mater Med. 2010;21(3):855–61. doi:10.1007/s10856-009-3914-y.

22. Sculean A, Nikolidakis D, Schwarz F. Regeneration of periodontal tissues: combinations of barrier membranes and grafting materials—biological foundation and preclinical evidence: a systematic review. J Clin Periodontol. 2008;35(8 Suppl):106–16. doi:10.1111/j.1600-051X.2008.01263.x.

23. Fatimi A, Tassin JF, Quillard S, Axelos MA, Weiss P. The rheological properties of silated hydroxypropylmethylcellulose tissue engineering matrices. Biomaterials. 2008;29(5):533–43.

24. Vinatier C, Magne D, Weiss P, Trojani C, Rochet N, Carle GF, et al. A silanized hydroxypropyl methylcellulose hydrogel for the three-dimensional culture of chondrocytes. Biomaterials. 2005;26(33):6643–51. doi:10.1016/j.biomaterials.2005.04.057.

25. Daculsi G, Uzel AP, Weiss P, Goyenvalle E, Aguado E. Developments in injectable multiphasic biomaterials. The performance of microporous biphasic calcium phosphate granules and hydrogels. J Mater Sci Mater Med. 2010;21(3):855–61. doi:10.1007/s10856-009-3914-y.

26. Struillou X, Boutigny H, Badran Z, Fellah BH, Gauthier O, Sourice S, Pilet P, Rouillon T, Layrolle P, Weiss P, Soueidan A. Treatment of periodontal defects in dogs using an injectable composite hydrogel/biphasic calcium phosphate. J Mater Sci Mater Med. 2011;22(7):1707–17. doi:10.1007/s10856-011-4344-1 Epub 2011 May 25.

27. Kilkenny C, Browne WJ, Cuthill IC, Emerson M, Altman DG. Improving bioscience research reporting: the ARRIVE guidelines for reporting animal research. J Pharmacol Pharmacother. 2010;1(2):94–9. doi:10.4103/0976-500X.72351.

28. Berglundh T, Stavropoulos A, Working Group 1 of the VIII European Workshop on Periodontology. Preclinical in vivo research in implant dentistry. Consensus of the eighth European workshop on periodontology. J Clin Periodontol. 2012;39(12 Suppl):1–5. doi:10.1111/j.1600-051X.2011.01827.x.

29. Schwarz F, Iglhaut G, Becker J. Quality assessment of reporting of animal studies on pathogenesis and treatment of peri-implant mucositis and peri-implantitis. A systematic review using the ARRIVE guidelines. J Clin Periodontol. 2012;39(12 Suppl):63–72. doi:10.1111/j.1600-051X.2011.01838.x.

30. Vignoletti F, Abrahamsson I. Quality of reporting of experimental research in implant dentistry. Critical aspects in design, outcome assessment and model validation. J Clin Periodontol. 2012;39(12 Suppl):6–27. doi:10.1111/j.1600-051X.2011.01830.x.

31. Bourges X, Weiss P, Daculsi G, Legeay G. Synthesis and general properties of silated-hydroxypropyl methylcellulose in prospect of biomedical use. Adv Colloid Interface Sci. 2002;99(3):215–28.

32. Struillou X, Boutigny H, Soueidan A, Layrolle P. Experimental animal models in periodontology: a review. Open Dent J. 2010;4:37–47. doi:10.2174/1874210601004010037.

33. Schwarz F, Sager M, Kadelka I, Ferrari D, Becker J. Influence of titanium implant surface characteristics on bone regeneration in dehiscence-type defects: an experimental study in dogs. J Clin Periodontol. 2010;37(5):466–73. doi:10.1111/j.1600-051X.2010.01533.x.

34. Zitzmann NU, Berglundh T. Definition and prevalence of peri-implant diseases. J Clin Periodontol. 2008;35(8 Suppl):286–91. doi:10.1111/j.1600-051X.2008.01274.x.

35. Renvert S, Roos-Jansåker AM, Claffey N. Non-surgical treatment of peri-implant mucositis and peri-implantitis: a literature review. J Clin Periodontol. 2008;35(8 Suppl):305–15. doi:10.1111/j.1600-051X.2008.01276.x.

36. Claffey N, Clarke E, Polyzois I, Renvert S. Surgical treatment of peri-implantitis. J Clin Periodontol. 2008;35(8 Suppl):316–32. doi:10.1111/j.1600-051X.2008.01277.x.

37. Romanos GE, Weitz D. Therapy of peri-implant diseases. Where is the evidence? J Evid Based Dent Pract. 2012;12(3 Suppl):204–8. doi:10.1016/S1532-3382(12)70038-6.

38. Froum SJ, Froum SH, Rosen PS. Successful management of peri-implantitis with a regenerative approach: a consecutive series of 51 treated implants with 3- to 7.5-year follow-up. Int J Periodontics Restor Dent. 2012;32(1):11–20.

39. Chiapasco M, Zaniboni M. Clinical outcomes of GBR procedures to correct peri-implant dehiscences and fenestrations: a systematic review. Clin Oral Implants Res. 2009;20(Suppl 4):113–23. doi:10.1111/j.1600-0501.2009.01781.x.

40. Rakhmatia YD, Ayukawa Y, Furuhashi A, Koyano K. Current barrier membranes: titanium mesh and other membranes for guided bone regeneration in dental applications. J Prosthodont Res. 2013;57(1):3–14. doi:10.1016/j.jpor.2012.12.001.

41. Darby I. Periodontal materials. Aust Dent J. 2011;56(1 Suppl):107–18. doi:10.1111/j.1834-7819.2010.01301.x.

42. Xu C, Lei C, Meng L, Wang C, Song Y. Chitosan as a barrier membrane material in periodontal tissue regeneration. J Biomed Mater Res B. 2012;100(5):1435–43.

43. Shin SY, Park HN, Kim KH, Lee MH, Choi YS, Park YJ, Lee YM, Ku Y, Rhyu IC, Han SB, Lee SJ, Chung CP. Biological evaluation of chitosan nanofiber membrane for guided bone regeneration. J Periodontol. 2005;76(10):1778–84.

44. Kuo SM, Chang SJ, Chen TW, Kuan TC. Guided tissue regeneration for using a chitosan membrane: an experimental study in rats. J Biomed Mater Res A. 2006;76(2):408–15.

45. Teng SH, Lee EJ, Wang P, Shin DS, Kim HE. Three-layered membranes of collagen/hydroxyapatite and chitosan for guided bone regeneration. J Biomed Mater Res B. 2008;87(1):132–8. doi:10.1002/jbm.b.31082.

46. Teng SH, Lee EJ, Yoon BH, Shin DS, Kim HE, Oh JS. Chitosan/nanohydroxyapatite composite membranes via dynamic filtration for guided bone regeneration. J Biomed Mater Res A. 2009;88(3):569–80. doi:10.1002/jbm.a.31897.

47. Jung RE, Hälg GA, Thoma DS, Hämmerle CH. A randomized, controlled clinical trial to evaluate a new membrane for guided bone regeneration around dental implants. Clin Oral Implants Res. 2009;20(2):162–8. doi:10.1111/j.1600-0501.2008.01634.x.

48. Dupraz A, Nguyen TP, Richard M, Daculsi G, Passuti N. Influence of a cellulosic ether carrier on the structure of biphasic calcium phosphate ceramic particles in an injectable composite material. Biomaterials. 1999;20(7):663–73.

49. Gauthier O, Bouler JM, Weiss P, Bosco J, Aguado E, Daculsi G. Short-term effects of mineral particle sizes on cellular degradation activity after implantation of injectable calcium phosphate biomaterials and the consequences for bone substitution. Bone. 1999;25(2 Suppl):71S–4S.

50. Gauthier O, Bouler JM, Weiss P, Bosco J, Daculsi G. Kinetic study of bone ingrowth and ceramic resorption associated with the implantation of different injectable calcium-phosphate bone substitutes. J Biomed Mater Res. 1999;47(1):28–35.

51. Schwarz F, Rothamel D, Herten M, Wüstefeld M, Sager M, Ferrari D, Becker J. Immunohistochemical characterization of guided bone regeneration at a dehiscence-type defect using different barrier membranes: an experimental study in dogs. Clin Oral Implants Res. 2008;19(4):402–15. doi:10.1111/j.1600-0501.2007.01486.x.

52. Polimeni G, Xiropaidis AV, Wikesjö UM. Biology and principles of periodontal wound healing/regeneration. Periodontol 2000. 2006;41:30–47.

53. Saffarzadeh A, Gauthier O, Bilban M. Bagot D'Arc M, Daculsi G. Comparison of two bone substitute biomaterials consisting of a mixture of fibrin sealant (Tisseel) and MBCP (TricOs) with an autograft in sinus lift surgery in sheep. Clin Oral Implants Res. 2009;20(10):1133–9. doi:10.1111/j.1600-0501.2009.01738.x.

54. Sculean A, Windisch P, Szendroi-Kiss D, Horvath A, Rosta P, Becker J, et al. Clinical and histologic evaluation of an enamel matrix derivative combined with a biphasic calcium phosphate for the treatment of human intrabony periodontal defects. J Periodontol. 2008;79(10):1991–9. doi:10.1902/jop.2008.080009.

55. Malard O, Guicheux J, Bouler JM, Gauthier O, de Montreuil CB, Aguado E, et al. Calcium phosphate scaffold and bone marrow for bone reconstruction in irradiated area: a dog study. Bone. 2005;36(2):323–30. doi:10.1016/j.bone.2004.07.018.

56. Jegoux F, Goyenvalle E, Cognet R, Malard O, Moreau F, Daculsi G, et al. Mandibular segmental defect regenerated with macroporous biphasic calcium phosphate, collagen membrane, and bone marrow graft in dogs. Arch Otolaryngol Head Neck Surg. 2010;136(10):971–8. doi:10.1001/archoto.2010.173.

57. Abushahba F, Renvert S, Polyzois I, Claffey N. Effect of grafting materials on osseointegration of dental implants surrounded by circumferential bone defects. An experimental study in the dog. Clin Oral Implants Res. 2008;19(4):329–34. doi:10.1111/j.1600-0501.2007.01455.x.

58. Herten M, Jung RE, Ferrari D, Rothamel D, Golubovic V, Molenberg A, Hämmerle CH, Becker J, Schwarz F. Biodegradation of different synthetic hydrogels made of polyethylene glycol hydrogel/RGD-peptide modifications: an immunohistochemical study in rats. Clin Oral Implants Res. 2009;20(2):116–25. doi:10.1111/j.1600-0501.2008.01622.x.