BMP-2 delivered from a self-cross-linkable CaP/hydrogel construct promotes bone regeneration in a critical-size segmental defect model of non-union in dogs

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Introduction

Despite the benefits that minimally invasive surgery and osteosynthesis have brought to fracture management and bone healing, there are still many circumstances where bone healing may remain challenging. Autologous bone grafts are still considered the gold standard in bone regeneration because of their osteogenicity, osteoinductivity, and osteoconductivity (1). However they also are associated with several disadvantages which result in significantly increased patient morbidity in humans (2). Calcium phosphate (CaP) bone substitutes such as hydroxyapatite (HA) and tricalcium phosphate (TCP), as well as mixtures of these compounds such as biphasic calcium phosphate (BCP) are currently used for bone substitution in many different clinical applications such as repair of bone defects after trauma or tumour, bone augmentation in spinal arthrodesis, periodontal treatment, or as coatings for metallic implants (3–6). They can also act as drug-delivery systems through their association with bioactive molecules that can be released in situ after implantation to favour bone regeneration (7). Although these bone substitutes are osteoconductive, they often lack the osteogenicity needed to support bone healing in large defects and are slowly degraded in the body.

Current strategies to promote non-union bone healing have been studied through different long-bone critical-size defect models with autologous bone grafts, bone-substitute biomaterials in combination with growth factors, and bone-marrow or tissue-engineered products with both autologous and allogenous mesenchymal stem cells (8–10). The lack of an ideal grafting option has spurred extensive research into the potential of growth factors including recombinant human bone morphogenetic proteins. Bone morphogenetic protein-2 (BMP-2) has been shown to accelerate bone healing in humans and animal models (7, 11–13). Bone morphogenetic protein-2 acts by osteoinduction and is involved in the differentiation of mesen-
chymal progenitor cells into osteoblasts (7, 11, 13). However, due to its rapid diffusion and degradation, appropriate delivery systems or carriers are necessary to prevent its dispersion from the site of application and achieve gradual release. Various carriers have been examined for bone morphogenetic proteins, such as bone matrix and collagen, synthetic polymers like poly(glycolic-co-lactic acid), and ceramic materials such as hydroxyapatite and beta-tricalcium phosphate (7, 12). A self-crosslinking polymer based on silanized hydroxypropyl-methylcellulose (Si-HPMC) has been developed and may act as a potential scaffold for bone regeneration (14). This hydrogel exhibits viscous and elastic properties that make its injection smooth, and its combination with BCP particles gives consistency to the product by linking granules together and improving their stability in the osseous defects. Depending on the size of the associated BCP granules, this association can provide injectable or mouldable hydrogel/BCP constructs that have been tested for use in both orthopaedic and dental surgery (14–16).

In human medicine, recent meta-analysis review has highlighted a paucity of data on the use of bone morphogenetic proteins in fracture healing, as well as considerable industry involvement in currently available evidence, and reported that the use of bone morphogenetic proteins for treating non-union remained unclear (17, 18). Only a few veterinary studies and clinical reports have been published (13, 19–25). Although the combination of BMP-2 with collagen sponges has proved to be a promising strategy for bone healing applications, collagen matrix may still have drawbacks in ensuring suitable BMP-2 in situ delivery, such as loss and leakage of the BMP-2 solution, insufficient affinity for the BCP-2 binding sites, inadequate porosity of the sponges, or collagen degradation related to a variety of sterilization processes (6). Recent studies investigated the association of BMP-2 with different biopolymers for BMP-2 retention and delivery and reported variable results depending on the BMP-2 source (26, 27). In this context, the purpose of this study was to determine whether the addition of BMP-2 to a Si-HPMC hydrogel/BCP granules construct could improve bone healing in critical-size ulnar defects in dogs, compared to the same construct without any BMP-2, and compared to autologous cancellous bone grafts.

**Materials and methods**

**Experimental design**

This study was conducted on 10 adult spayed female Beagle dogs (11 ± 1.5 kg; 8 ± 1 year old) after approval by the Pays de Loire Animal Ethical Committee (No. CEEA.2012.120) and the Oniris College of Veterinary Medicine Animal Welfare Committee. Haematology was performed preoperatively to ensure the absence of coagulopathy and systemic diseases. The dogs had no signs of bone or joint disease on the relevant limbs, as assessed by clinical examination and preoperative radiographs.

**Implant preparation**

The hydrogel/BCP constructs were prepared immediately before their use in surgery, under aseptic conditions. The macroporous BCP ceramic granules were 0.5 to 1 mm in diameter with a HA/β-TCP weight ratio of 60/40. The hydrogel was a 3% aqueous solution of a cellulosic derivative polymer (HPMC) of which the biocompatibility had already been demonstrated (14). Grafting of silanol groups onto the HPMC polymer was performed using a previously published method that provides self-crosslinking properties to the hydrogel through a non-exothermic acid-base reaction (14, 15). The lyophilisate recombinant human bone morphogenetic protein 2b (rhBMP-2) (0.33 mg/implant) was solubilised with the aqueous phase of the hydrogel. Then, the BCP granules were mixed with the Si-HPMC hydrogel in a 40/60 w/v ratio to form a mouldable hydrogel/BCP scaffold with or without any rhBMP-2.

**Anaesthesia**

For each dog, a fentanyl patch (5 μg/kg) was applied to the skin on the lateral flank 12 hours prior to surgery. Morphine hydrochloride (0.2 mg/kg, SC) and acepromazine (0.05 mg/kg, SC) were administered for premedication. Anaesthesia was induced with propofol (6 mg/kg, IV) and maintained with isoflurane in 100% oxygen. Cefalexin (30 mg/kg IV) was administered after general anaesthesia was induced. No postoperative antibiotic treatment was given.

**Surgical procedure**

With the animal positioned in lateral recumbency, a skin incision was made to perform a caudo-lateral approach of the ulna. Measurements of the distance from the olecranon to the styloid process were made from preoperative radiographs and reported perioperatively to ensure reproducibility of the defect at a standard location. A bilateral standardized 2 cm long diaphyseal ulnar osteotomy, including the enveloping periosteum, was performed in five dogs, using an oscillating saw under constant saline irrigation (28, 29). Stabilization of the proximal and distal bone ends was achieved with an eight-hole 2.4 locking bone plate with three and two locking screws inserted into the proximal and distal ulnar fragments respectively. During surgery, a hydrogel/BCP construct with rhBMP-2 was implanted into the bone defect on one side and a hydrogel/BCP construct without any rhBMP-2 was implanted into the contralateral limb so that each animal was its own control. The BCP granules were easy to handle when associated with the hydrogel, and the constructs fitted the defect dimensions perfectly. As positive controls, five additional dogs had a unilateral ulnar ostectomy that was filled with autolo-

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**References**

1. Graftys, Aix-en-Provence, France
2. Truscient: Zoetis Belgium, Louvain-la-Neuve, Belgium
3. Durogesic: Janssen-Cilag, Beerse, Belgium
4. Morphine chloride: CDM Lavoisier, Paris, France
5. Calmivet: Vetoquinol, Lure, France
6. Propolipid: Fresenius Kabi, Sèvres, France
7. Vethetane: Virbac, Carros, France
8. Riflaxine: Virbact SA, Carros, France
9. LCP: Synthes, Lure, France

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gous cancellous bone harvested from the ipsilateral proximal humerus. The surgical wounds were closed routinely.

**Postoperative care**

Postoperative pain was assessed every two hours during the first 24 hours and then every six hours for the following five days and controlled with morphine injections when necessary. Meloxicam® (0.1 mg/kg) was administered orally during the first five postoperative days. The animals were kept in individual cages with modified Robert-Jones bandages applied to the treated fore-limbs during the first three postoperative days. Any abnormality, such as swelling or discharge from the incision site, or persistent lameness, was noted in the medical record.

**Radiological assessment**

Cranio-caudal and mediolateral radiographic views of each osteotomy site were assessed immediately after surgery to monitor the position of the implants, and at four, eight, 12, 16 and 20 weeks postoperatively to assess union with the host bone at both proximal and distal junctions. Bone union (BU) and bone formation (BF) were also assessed for the rhBMP-2-treated and untreated constructs and the autologous cancellous bone grafts according to a previously described scoring system, in which proximal and distal junctions were both graded from 0 to 3 for bone union (BU) and from 0 to 4 for bone formation (BF) at the junctions (30). Thus the highest possible score was 6 for BU and 4 for BF. The combined score (BU+BF) refers to the sum of the scores for BU and BF; the maximum score being 10. Resorption of the implant was assessed only qualitatively.

**Histological analysis**

Autografts, and rhBMP-2-treated and untreated specimens were then fixed in 10% formalin solution and prepared for histological analysis by both light microscopy and scanning electron microscopy (SEM) to assess bone union at the junctions and new bone formation within the constructs. Undecalcified specimens were dehydrated in graded ethanol series and pure acetone. The samples were impregnated with glycolmethacrylate for seven days and then embedded in a glycolmethylmethacrylate resin. The samples were sectioned longitudinally, polished and then sputter-coated with carbon and observed by SEM. Images were acquired using the back-scattered electron mode. Light microscopy was performed on 5 µm thick sections stained with Movat’s pentachrome. Sections were obtained parallel to the long axis of the bone and extending over the length of the defect. Both the radiographic and histological assessments were blindly performed.

**Microcomputed tomography**

Twenty weeks after implantation, the constructs were explanted during a second surgical procedure, preserving both proximal and distal interfaces and implants were removed. Specimens were conserved frozen until microcomputed tomography (µCT) imaging was performed. Images were acquired under an 80 keV voltage with a constant 500 µA current. The slice thickness was 110 µm and each acquisition generated 512 images with a pixel size of 110 µm.

**Results**

**Clinical results**

The clinical outcome was considered excellent in all cases and weight-bearing usually started during the first postoperative day. The implanted biomaterials did not cause any apparent signs of irritation or infection, and cutaneous wound healing was uneventful.

**Radiographic results**

All the proximal junctions and four out of five of the distal junctions appeared healed at eight weeks after implantation with the rhBMP-2-treated constructs (Figure 1, Table 1), while only one proximal junction and none of the distal junctions appeared healed with the untreated constructs (Figure 2, Table 1). No signs of implant loosening or pseudarthrosis inside the implantation site were detected during the radiological follow-up. In the defects filled with bone autografts, all but one proximal and one distal junction exhibited bone union at 20 weeks (Figure 3, Table 1, Table 2). Bone healing at the junctions appeared to be delayed after implantation for untreated constructs compared to rhBMP2-treated ones (Figure 1, Table 1, Table 2). The scores for BU, BF, and BU+BF at four, eight, 12 and 20 weeks, as assessed from radiographs, are shown in Table 2. The BU, BF, and BU+BF scores of rhBMP-2-treated constructs were significantly higher than the scores for the untreated ones at eight, 12, and 20 weeks.

Neither osteointegration nor resorption of the ceramic granules could be precisely assessed on the radiographs for both groups as the BCP particles radiodensity prevented accurate radiographic evaluation of bone ingrowth.

In one dog, the hydrogel was not sufficiently set at the time of implantation, and some BCP granules and hydrogel treated with rhBMP-2 leaked outside the defect area. Eight weeks later, bone union was achieved and the defect was filled with new

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**Table 1.** The BU, BF, and BU+BF at four, eight, 12 and 20 weeks.

<table>
<thead>
<tr>
<th>Time (weeks)</th>
<th>BU (0-3)</th>
<th>BF (0-4)</th>
<th>BU+BF (0-10)</th>
</tr>
</thead>
<tbody>
<tr>
<td>4</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>8</td>
<td></td>
<td></td>
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<td>12</td>
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<tr>
<td>20</td>
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</tr>
</tbody>
</table>

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**Table 2.** Bone healing at the junctions.

<table>
<thead>
<tr>
<th>Junction</th>
<th>BU</th>
<th>BF</th>
<th>BU+BF</th>
</tr>
</thead>
<tbody>
<tr>
<td>Proximal</td>
<td>6</td>
<td>4</td>
<td>10</td>
</tr>
<tr>
<td>Distal</td>
<td>4</td>
<td>4</td>
<td>8</td>
</tr>
</tbody>
</table>

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k Inveon µCT: Siemens Preclinical Solutions, Knoxville, TN, USA
l Leo 1450VP: Zeiss, Oberkochen, Germany
m Graph PadPrism v6 software: GraphPad Software, San Diego, CA, USA
bone tissue despite the perioperative leakage. However, extensive heterotopic bone formation was also observed within the soft tissues surrounding the defect area, from the implantation site to the distal end of the ulna (Figure 4).

During explantation, the gross findings for the implanted sites were in accordance with the radiographic images. In the rhBMP-2-treated constructs, the plates were covered with various amounts of firm bone, without invasion of surrounding soft tissue. In contrast, in the untreated constructs, there was minimal evidence of bone union at both the proximal and distal interfaces between the various implanted materials and the host bone.

### Table 1

<table>
<thead>
<tr>
<th></th>
<th>8 weeks postoperatively</th>
<th>20 weeks postoperatively</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>rhBMP-2-treated constructs (n = 5)</td>
<td>Untreated constructs (n = 5)</td>
</tr>
<tr>
<td>Bone union at proximal interface</td>
<td>5</td>
<td>1</td>
</tr>
<tr>
<td>Bone union at distal interface</td>
<td>4</td>
<td>None</td>
</tr>
</tbody>
</table>

rhBMP-2 = recombinant human bone morphogenetic protein 2.

### Figure 1

Medio-lateral radiographs of an ulnar defect filled with the hydrogel/BCP construct with rhBMP-2: a) immediately after surgery, and at b) eight weeks, c) 12 weeks, and d) 20 weeks postoperatively. New bone formed within the first eight weeks of implantation, without any noticeable change until 20 weeks. There was also development of new cortex at the cranial edge of the defect from eight weeks postoperatively.

### Figure 2

Medio-lateral radiographs of an ulnar defect filled with the hydrogel/BCP construct without any rhBMP-2: a) immediately after surgery, and at b) eight weeks, c) 12 weeks, and d) 20 weeks postoperatively. Radiodense BCP particles remained inside the defect area due to the cross-linking properties of the hydrogel, but the formation of new bone was difficult to assess, even though the proximal junction was considered to show signs of bone union at 12 weeks postoperatively.
tissues, but all the metallic implants could be easily removed. All untreated constructs exhibited a soft tissue consistency.

**MicroCT results**

MicroCT assessment was able to distinguish between the three phases present inside the defects: newly formed bone, remaining BCP ceramic and non-mineralized tissues. Assessment of bone healing at proximal and distal junctions remained uncertain, even on three-dimensional reconstructed images. The rhBMP-2-treated constructs seemed to show a smaller quantity of remaining BCP particles and more obvious new-bone colonisation than the untreated ones.

**Histological results**

Qualitative assessment was made of the amount of remaining ceramic within the defect and of the organization and maturity of regenerated bone.

On SEM investigation, bone union was observed at all the proximal and distal interfaces where the defects had been filled with the rhBMP-2-treated constructs, whereas only one of the proximal junctions and three of the distal ones where the untreated constructs had been used showed bone union. The untreated constructs showed a larger remaining quantity of BCP particles, a limited amount of newly formed bone at the junctions and an absence of bone formation in the centres of the defects.

Conversely, the rhBMP-2-treated constructs showed a residual amount of BCP particles and abundant new lamellar bone formation that had developed in direct contact with BCP particles without any fibrous interface. The new bone network showed many osteocytic lacunae and growth both on the BCP granule surfaces and in the intergranular spaces.

**Table 2** Median and range of radiographic scores for bone union (BU, max 6) and bone formation (BF, max 4); and the combined score (BU+BF, max 10), scored according to Johnson et al. (30).

<table>
<thead>
<tr>
<th>Weeks</th>
<th>rhBMP-2-treated constructs</th>
<th>Untreated constructs</th>
<th>Autologous cancellous bone</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>BU</td>
<td>BF</td>
<td>BU+BF</td>
</tr>
<tr>
<td>4</td>
<td>1a (0–3)</td>
<td>1a (0–2)</td>
<td>3a (0–3)</td>
</tr>
<tr>
<td>8</td>
<td>5a (4–6)</td>
<td>2a (2–3)</td>
<td>7a (6–9)</td>
</tr>
<tr>
<td>12</td>
<td>5a (5–6)</td>
<td>3a (2–3)</td>
<td>8a (7–9)</td>
</tr>
<tr>
<td>20</td>
<td>6a (5–6)</td>
<td>3a (2–4)</td>
<td>8a (7–10)</td>
</tr>
</tbody>
</table>

Within each row, values for each parameter (BU, BF or BU+BF) that do not share the same superscript letters are significantly different. *p <0.05.
surrounded by a dense, non-mineralized connective tissue (▶Figure 10). For autologous cancellous bone grafts in control dogs, microCT, SEM and conventional histology confirmed the bone healing of the defects as observed on radiographs (▶Figure 3, ▶Figure 5C, ▶Figure 6C, ▶Figure 11).

**Discussion**

This study has described the combination of a self-crosslinkable hydrogel and CaP-particle bone substitute to locally deliver rhBMP-2 for bone regeneration at critical-size ulnar defects. The rhBMP-2-treated constructs encouraged the formation of abundant bone tissue bridging both proximal and distal interfaces as early as eight weeks after surgery, without any noticeable changes until 20 weeks. Both microCT and SEM imaging techniques are particularly suitable ones to describe the in vivo behaviour of CaP biomaterials. Together with conventional light microscopy histology, such techniques brought more reliable information about bone healing in such defect models than radiographs.

Newly formed bone tissue within the implantation site was well-differentiated mineralized lamellar bone surrounding the BCP granules, as usually observed with such CaP biomaterials. The rhBMP-2 release from the constructs did not alter the quality, the architecture and the mineralization of the newly formed bone, as shown by the number of osteocytic lacunae and Haversian systems and the presence of an osteoid border at the surface of the new trabeculae. Constructs without any rhBMP-2 showed osteoconductive properties limited to the bone junctions and no bone ingrowth into the implantation site, showing a lack of osteoinduction. The results obtained with the autografts were similar to the ones from the rhBMP-2 constructs at 20 weeks, but radiographically, bone regeneration at the junctions between the host cortical bone and the implanted biomaterials appeared to develop earlier with the rhBMP-2-treated constructs than with the bone autografts.

Regeneration to compensate for bone defects can be accelerated by localized delivery of appropriate growth factors.
incorporated within a biodegradable carrier. The carrier may allow the impregnated growth factor to release at a desirable rate and concentration, and can be formulated to have a particular structure facilitating cellular infiltration and growth (31, 32).

In our study, both Si-HPMC hydrogel and BCP granules confirmed to be safe and biocompatible biomaterials, with favourable rheological and biological properties (14, 16, 33, 34). The addition of rhBMP-2 to hydrogel/BCP constructs improved bone healing at 20 weeks, compared to the hydrogel/BCP combination alone that did not display significant osteoconductive ability. The addition of the hydrogel to the BCP granules made them easier to manipulate and may have enhanced their initial stability within the defect area. In the case where the biomaterial leaked outside

Figure 6 Microtomography three-dimensional images of a defect filled with the hydrogel/BCP construct: a) with rhBMP-2, b) without any rhBMP-2, and c) with an autologous cancellous-bone graft 20 weeks postoperatively.

Figure 7 SEM images of bone defects filled with the rhBMP-2-treated hydrogel/BCP construct, 20 weeks postoperatively. BCP particles (*) appear white, bone tissue grey, and non-mineralized soft tissues black. a) New bone formation appears denser in the cortex area than in the medullary cavity area. b) Higher magnification SEM image at the cortical junction with the host bone, showing that the new bone tissue developed in direct contact with the BCP surface and joined the BCP particles together. c) Many Haversian systems are observed within the newly formed bone with numerous osteocytic lacunae (blue arrows) in the new bone lamellae.
Figure 8  SEM images of a bone defect filled with a construct without any rhBMP-2 at 20 weeks postoperatively. a) Longitudinal median section showing bone cortical and marrow cavity. The implant bent during the histology preparation due to the absence of new bone colonization. b) Higher magnification of the proximal bone-implant junction. New bone formation developed on the surface and between the BCP particles only in the immediate contact area with the host bone. No bone formation was observed in the centre of the defect. c) Higher magnification of the distal bone-implant junction with very limited osteoconduction.

Figure 9  Histological microphotograph of a defect filled with a rhBMP-2-treated hydrogel/BCP construct, 20 weeks postoperatively. a) A matrix of newly formed bone developed between the BCP particles (*) and in the unfilled holes of the LCP plate (arrows). Newly formed bone appears yellow and the remaining BCP particles light blue (Movat’s pentachrome staining). b) Higher magnification image at the junction with the host bone cortex. BCP particles are surrounded by abundant, well-differentiated and mineralized new bone formation. c) Higher magnification image of the medullary cavity area. The newly formed bone, which appears active as an osteoid border (in red), was observed at the surface of each new bone trabeculae. c: cortical bone, mc: marrow cavity.
the implantation site, it was responsible for extensive ectopic ossification along the distal ulna diaphysis, emphasizing that controlled local release of the rhBMP-2 in the immediate vicinity of the implantation site remained the most critical point. Such a crosslinkable biomaterial proved to be able to contain both the BCP particles and the associated active molecule within the implantation site and its association with a BCP osteoconductive matrix supported the sustainable development of a new lamellar bone network. This polymer has already shown potentially useful properties in periodontal applications, where the hydrogel enhances intergranular cohesion and supports the formation of new bone, but also seems to act as an exclusion barrier preventing epithelial growth between the granules (15, 16). Similarly, in the present study, the presence of the hydrogel in the constructs without any rhBMP-2 may have prevented early cell colonization and subsequent bone ingrowth in soft tissue situations where osteoconduction was limited to bone junctions.

Numerous published studies have shown BMP-2 to have the highest osteoinductive potential among all the bone morphogenetic proteins. There are differences between species in their sensitivity to bone morphogenetic proteins, so the results can be difficult to extrapolate from one species to another (36). A minimum threshold dose of BMP-2 is necessary for a beneficial effect to occur, but a higher dose does not necessarily result in a better outcome (11, 37). Increasing the BMP-2 dose has been reported to increase cyst-like bone voids and reduce biomechanical values (11, 37). A high dose of BMP-2 also seems to induce a larger amount of ectopic bone in surrounding tissues and has the potential to disturb vital structures such as nerves or muscles, possibly leading to discomfort or pain (17, 18). Reducing the bone morphogenetic protein dose required for bone repair would be desirable from both safety and economy viewpoints. Previous studies have tried to investigate the outcomes from using various concentrations of rhBMP-2, ranging from 0.1 mg to more than 0.9 mg per implant (11, 20, 36, 38, 39). It appears difficult to decide on an ideal concentration, since these studies varied in terms of the BMP-2 carrier and the size of the bone defect. In our study, the dose of 0.33 mg of rhBMP-2 from an available veterinary product added to the hydrogel/BCP construct allowed complete bone regeneration of diaphyseal critical-size defects. In human surgery, the main concerns regarding the use of bone morphogenetic proteins are ectopic bone formation due to in situ supraphysiological dose release, un-

Figure 10 Histological microphotograph of a defect filled with the hydrogel/BCP construct, without any rhBMP-2, 20 weeks postoperatively (Movat's pentachrome staining) (same implant as in Figure 8). a) A large amount of BCP particles (*) remained within the implantation site, surrounded by dense, non-mineralized connective tissue (red colour). b) Higher magnification at the junction with the host bone with very limited new bone apposition on the BCP particles in the immediate vicinity of the host bone (arrow). c) Higher magnification of the centre of the defect. A dense cellular and non-mineralized connective tissue developed around the BCP particles. c: cortical bone, mc: marrow cavity.

Figure 11 Histological microphotograph of a defect filled with autologous bone graft, 20 weeks postoperatively (Movat's pentachrome staining) showing complete healing of the defect from proximal (arrow) to distal junction and restoration of both bone cortices and medullary cavity. c: cortical bone, mc: marrow cavity.
predictable results (particularly for treatment of non-union), cost-effectiveness, and industrial pressure (4, 18). The carrier, delivery profile and appropriate dose all have still to be optimized (7, 18, 40, 41). In the present study, differences between rhBMP-2-treated and untreated implants were seen on radiographs as early as four to eight weeks postoperatively. These results could be explained by the fact that rhBMP-2 is probably rapidly released after implantation. Previous studies have shown that bone formation occurs 28 days after implantation of BMP-2-impregnated collagen sponges and that 0.5% of the initial dose of BMP-2 remained after 21 days (42, 43). Conversely, a 4 μg/mL rhBMP-2-treated chitosan-based hydrogel retained about 99% of the encapsulated rhBMP-2 even after 30 days of incubation in vitro (44). Consequently, as the nature of the delivering scaffold appears to be a key factor controlling the local release of bioactive molecules, further studies remain needed to assess the kinetic release of rhBMP-2 from our hydrogel/BCP construct. The ulnar ostectomy model we used allowed bilateral implantations during the same procedure, with minimal adverse functional consequences. Previous studies in dogs have shown that a 2 cm osteotomy corresponds to a critical-size defect that does not heal spontaneously without the addition of a bone graft or bone substitute (28, 40, 45, 46). Such an experimental model of non-union provides very limited osteoconductivity as bone contact was only present at both proximal and distal cortical junctions. The rhBMP-2 appeared to have osteoinductive properties that were quite efficient in such surgical conditions, and combining this material with a CaP matrix and a crosslinkable hydrogel ensured its controlled local release, together with earlier bone healing as compared to autologous bone grafts, and resorption–bone substitution of the BCP granules.

Limitations

Such an experimental non-union model remained very different from clinical situations but prospective case-controlled clinical trials may be difficult to standardize as each clinical case may be influenced by individual and specific pathological conditions.

The ideal artificial-graft material should have osteogenicity, osteoinductivity, and osteoconductivity similar to those of a cancellous-bone graft. Without any rhBMP-2, the untreated constructs did not display any osteoinductive or significant osteoconductible activity. The ideal bone graft material should also have a rapid resorption rate to facilitate radiographic assessment (30). The presence of a radiodense ceramic may, however, partially interfere with radiographic assessments. The low resorption rate at 20 weeks may also mask non-union or call into question the long-term persistence of such bio-materials. Additional investigations may be needed to quantitatively investigate resorption of the CaP matrix, and the final mechanical properties and long-term stability of the regenerated bone. Furthermore, the addition of another control group with BCP granules alone would also allow assessment of the effect of the gel.

Conclusion

The findings of this study shows that the addition of rhBMP-2 to CaP ceramics in a self-crosslinkable hydrogel could promote bone regeneration in a critical-size-defect model controlled study. Such a combination could be a good choice for promoting bone regeneration in large bone defects, and be a step towards an ideal synthetic bone substitute with performance that is similar to autologous bone grafts. Further studies remain necessary, to further improve setting of the gel and avoid any ectopic bone formation related to leakage of the biomaterial and of the active substance outside the implantation site.

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Conflict of interest

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