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Native bovine bone morphogenetic protein improves the potential of biocoral to heal segmental canine ulnar defects

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Abstract We studied the effect of a composite implant consisting of coral and native bovine bone morphogenetic protein (BMP) on the healing of 2 cm segmental defects in the canine ulna. Plain coral and cortical autograft bone implants were used as controls. The fixation was temporary for 9 weeks with an intramedullary Kirschner wire (6 ulnas with a composite implant of coral and BMP, 6 with plain coral and 6 with an autograft) or a plate and screws (3 ulnas with a composite implant and 3 with plain coral). X-rays were taken at 3, 6, 9, 12, 16, 26 and 36 weeks, and mechanical torsion tests were performed at the end of the study. The score for bone formation and bone union evaluated from radiographs was significantly higher in the composite implant group than in the plain coral group at 16 weeks, but the score was even higher with autografts. BMP accelerated the resorption of the coral implant. The mechanical strength of the composite implants was higher than that of the bones with a plain coral implant ($P<0.05$), while the mechanical strength of the coral implants, even with BMP, was significantly lower than the strength of autografts ($P<0.01$). In conclusion, BMP enhanced the capacity of a coral implant to heal a segmental ulnar defect by increasing bone formation, but the effect of this combination was not as good as that of a cortico-cancellous autograft.

Résumé Nous avons étudié l'effet d'un implant composite, fait de corail imprégné de protéine de la morphogenèse osseuse (BMP) bovine, sur la consolidation d'une perte de substance de 2 cm de l'os ulnaire canin. Des implants comprennent du corail simple ou des autogreffes d'os cortical étaient employées comme contrôles. Une fixation temporaire intramedullaire de 9 semaines était réalisée, soit avec des broches de Kirschner (6 os ulnaires avec un implant composite, 6 avec du corail simple, et 6 avec une autogreffe), soit avec une plaque-à-vis (3 avec un implant composite et 3 avec du corail simple). Des radiographies étaient prises 3, 6, 9, 12, 16, 26 et 36 semaines aprés la mise en place des implants, et des tests mécaniques de torsion étaient réalisées en fin d'étude. Aprés 16 semaines, les taux de formation et d'union osseuses étaient significativement plus élevés avec les implants composites qu'avec les implants de corail simple, mais des taux encore plus élevés étaient observés dans les essais avec les autogreffes. La BMP a accéleré la résorption de l'implant de corail. La résistance mécanique des os avec un implant composite était plus forte que celle des os ayant un implant de corail simple ($P<0.05$), alors que la résistance mécanique des os traités par une autogreffe était plus forte ($P<0.01$) que celle des os ayant un implant de corail, avec ou sans BMP. En conclusion, la BMP améliore le pouvoir de consolidation des implants de corail qui reste cependant inférieur à celui observé avec les autogreffes.

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Introduction

Autogenous bone graft has been considered the gold standard for bone-repairing procedures as it contains the triggering ingredients necessary for bone formation in cases of bone defect. However, the availability of autograft bone is limited, and the harvesting of autograft bone causes morbidity at the donor site. Allografts have become common alternatives to autografts. However, allografts have disadvantages, such as the extended incorporation time and the possibility of disease transmission. Therefore, alternative bone substitute materials have been developed.

Coral has been used as a bone substitute in many experimental studies [1, 6, 10–12, 22, 23]. It has been proven to be biocompatible, biodegradable, and easy to handle [10–12, 19, 26], and it has not been found to cause any inflammatory responses [1,11]. However, it is not osteoinductive in itself.

Bone morphogenetic proteins (BMP) are a large family of non-collagenous proteins, which are osteoinductive, i.e. able to produce bone at ectopic sites [25]. BMPs initiate chondroplastic differentiation in pluripotent mesenchymal progenitor cells, followed by synthesis of new bone by enchondral ossification [27]. The effect of BMP on bone induction in experimental animals has been demonstrated in many studies [2–5, 9, 13, 16, 18,20, 21, 23, 28, 29]. BMP needs a delivery system to be successfully active in the target tissue [14].

In this study we compared fresh autografts, biocoral implants and composite implants, consisting of biocoral and bovine native BMP in the healing of canine ulnar defects. The aim of this study was to evaluate the coral implant as a carrier material of BMP and the effect of BMP on the capacity of coral implant to heal a canine ulnar defect.

Materials and methods

Laboratory-bred beagle dogs, both male and female, aged 1 year and weighing 9.0–13.2 kg, were used. The committee on animal experimentation of Kuopio University approved all experiments.

Cylindrical plain coral implants (Biocoral, Inoteb, Saint-Gonnery, France), about 9 mm in diameter and 20 mm in length, were used in 6 dogs (group Cor-K) and composite implants containing coral and native bovine BMP were used as ulnar transplants in another group of 6 dogs (Cor+BMP-K) (Fig. 1). The implants were predrilled longitudinally to produce a medullary canal. The BMP was extracted from bovine diaphyseal bones as described earlier [8]. This partially purified BMP including a combination of several growth factors was used at a dose of 30 mg per implant, and BMP was adsorbed into a collagen sponge (Lyostypt, Braun-Melsungen AG), which was wrapped around the coral cylinder. Similar implants were used in 3 more dogs with plate fixation (groups Cor-P and Cor+BMP-P). The implants were sterilized with ethylene oxide. Fresh ulnar cortical autografts were used as controls (autograft group). Table 1 shows the groups in this study.

The operations were performed with the dogs under general anesthetic by using pentobarbital (Mebunat, Orion-Farmos, Helsinki, Finland) at a dose of 15 mg/kg intravenously up to effect. Xylazine (Rompun Vet, Bayer, Germany) at 1 mg/kg was used as premedication before the operation. For the operation, forelegs were prepared and draped in a sterile fashion. A rubber band was used as a tourniquet above the elbow joint. A lateral incision was made and the ulna exposed. Using an oscillating saw, an osteotomy including periosteum was made in mid-ulna and a 2 cm defect was inflicted.

A Kirschner wire (thickness 1.2 mm) introduced into the medullary canal through the tip of olecranon and extending about 3 cm distally to the distal end of the implant was used for intramedullary fixation (groups Cor-K, Cor+BMP-K and Autograft, see Table 1). The Kirschner wires were removed after 9 weeks.

Plate fixation was performed with a 10-hole stainless steel miniplate and screws (Stratec Medical, Oberdorf, Switzerland). In dogs with plate fixation, plain coral implants were used in the left ulna and coral+BMP in the right (groups Cor-P and Cor+BMP-P, respectively, see Table 1).

The pain medication after the operation consisted of buprenorfin (Temgesic, Reckitt & Colman, Hull, UK) at 0.01 mg/kg intramuscularly. The dog chow was Serti (Suomen Nestle, Helsinki, Finland). The dogs were kept in separate cages for 1–2 days after the operation and thereafter in large outdoor/indoor runs with shelter for the duration of the study.

The dogs with Kirschner wire fixation were killed after 36 weeks and those with plate fixation after 16 weeks with an overdose of pentobarbital (Mebunat, 60 mg/kg intravenously). The ulnas were dissected and the soft tissue removed. The bones were wrapped in saline and frozen at –20°C until the analysis.

Radiography

After operation the positions of the implants were checked radiographically (Fig. 1). Bone healing was evaluated with further X-rays taking antero-posterior and lateral views at 3, 6, 9, 12, 16,

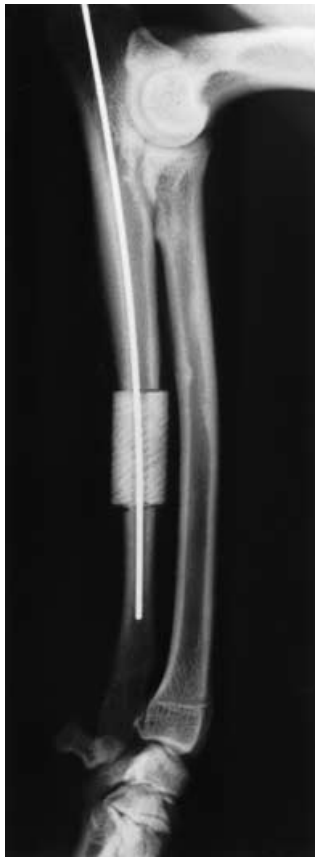


Fig. 1 Post-operative X-ray radiograph showing a coral implant fixed with an intramedullary Kirschner wire in a 2 cm ulnar defect

Table 1 Study groups (*K* Kirschner wire, *P* plate and screws)

	Cor-K	Cor-P	Cor+BMP-K	Cor+BMP-P	Autograft
Number of ulnas	6	3	6	3	6
Implant	Coral	Coral	Coral+BMP	Coral+BMP	Autograft
Fixation	K	P	K	P	K
Follow-up time (weeks)	36	16	36	16	36

25 and 36 weeks. Bone union, callus formation, and resorption of the implant was estimated independently by 2 investigators. Any cases of disagreement were reviewed together. The interpretation was blinded between the different study groups.

In the evaluation of bone union (BU) we used the scoring system proposed by Johnson et al. [17], in which proximal and distal union sites were both graded from 0–3. Thus, the highest possible score for bone union was 6. Bone formation (BF) was also scored, the maximum score being 4 [17]. The combined score (BU+BF) refers to the sum of the scores for bone union and bone formation, the maximum score being 10. The resorption of the coral implant was evaluated by scoring it as 0–3, as described in Table 2.

Mechanical testing

The ulnas were thawed at room temperature for torsional testing. During the testing the bones were kept moist [24]. The bone ends were embedded into moulds with two-component fiberglass resin, using a torsional shaft of 8 cm. After hardening of the resin, the bones were placed in the torque machine (Fig. 2) and torsionally loaded at a constant angular speed of 6.5°/s until failure [15]. Maximal torque capacity (MTC) was recorded.

Histology

After torsional testing a 4- to 5-cm-long section, including the implant site, was taken for histological analysis. After fixing in 10% neutral formaldehyde, the previously frozen samples were decalcified in 0.1 N HCl. The samples were embedded in paraffin, and 6 µm sections were stained with the Masson-Goldner trichrome method.

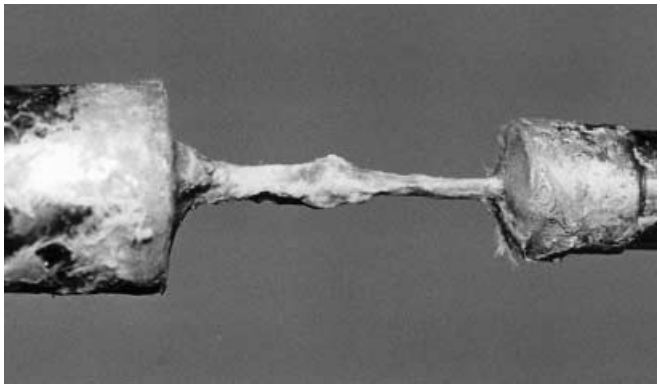


Fig. 2 An ulna attached for torsional testing

Table 2 Evaluation of the resorption of coral from radiographs

Score	Description
0	No resorption
1	Implant resorbed partially (<50%)
2	Implant resorbed partially (>50%)
3	Implant resorbed completely

Statistical analysis

A non-parametric Mann-Whitney test was used to compare the scores between the groups. Mann-Whitney test was also used in the analysis of the MTC because of the non-normality of the material. The values of unstable samples were replaced with the value zero in the analysis. Statistical analysis was performed using the SPSS for Windows statistical package (SPSS Inc., version 7.5.1). Values of P less than 0.05 were considered statistically significant.

Results

Generally, the dogs tolerated the operation well and weight-bearing normally started during the first postoperative day. The implants did not cause any noticeable irritation or infection in the forelegs. None of the Kirschner wires, but 4 of the 6 plates were broken.

Bone union and bone formation

The scores for bone union and bone formation at 16 and 36 weeks as evaluated from radiographs are shown in Table 3. Generally, the bone union caused by coral+BMP composite grafts was better than that caused by coral only, but not so comprehensive as with autografts. At the end of the study, there was 1 case in the group Cor-K with a nearly complete bridge of new bone at the implant site and 3 cases with no signs of union or bone formation. No signs of union were found in 2 cases in the group Cor-P. In the group Cor+BMP-K, 3 cases had nearly complete union. Two of the three ulnas in the group Cor+BMP-P showed acceptable bone union and marked bone formation (Fig. 3), but the third case with a broken plate was without any sign of union. In the autograft group, 4 of the 6 ulnar defects showed complete union.

Statistically a significant difference in the combined score for bone union and bone formation between the Cor-K and Cor+BMP-K groups was seen at 16 weeks, the score being higher for the BMP group (P=0.041). However, this significance disappeared at 36 weeks. The respective scores for autografts at 16 and 36 weeks were significantly higher in comparison with all the other groups, but not with the combined composite groups Cor+BMP-K and Cor+BMP-P at 16 weeks (Table 4).

Table 3 Mean scores for bone union (BU, max 6) and bone formation (BF, max 4) and the combined score (BU+BF, max 10) at 16 and 36 weeks, scored according to Johnson et al. [17]. The groups Cor-P and Cor+BMP-P were followed up for only 16 weeks

Week	Cor-K			Cor-P			Cor+BMP-K			Cor+BMP-P			Autograft		
	BU	BF	BU+BF	BU	BF	BU+BF	BU	BF	BU+BF	BU	BF	BU+BF	BU	BF	BU+BF
16	1.0	0.8	1.8	1.0	0.3	1.3	2.2	2.0	4.2	3.3	2.3	5.6	4.2	2.5	7.0
36	1.5	1.3	2.8				3.6	2.2	5.8				5.5	3.7	9.2

Table 4 *P*-values of the Mann-Whitney test of the scores for bone union (BU) and bone formation (BF) and the combined score (BU+BF). For the description of groups, see Table 1

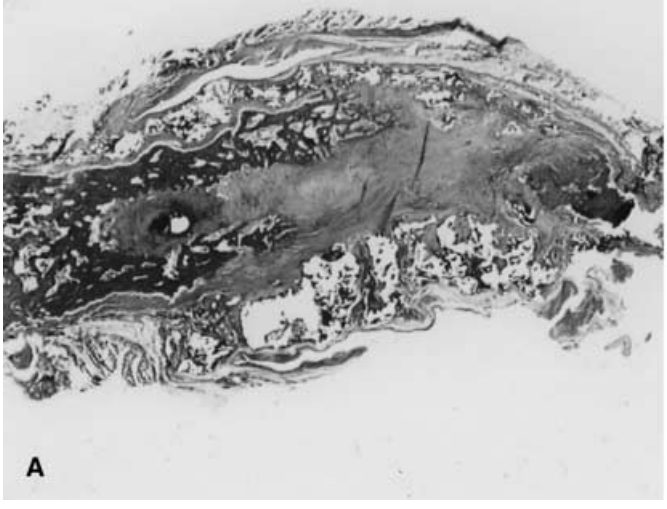
	n	BU	BF	BU+BF
Cor-K vs Cor+BMP-K				
16 weeks	6/6	0.093	0.026	0.041
36 weeks	6/6	0.093	0.240	0.132
Cor-K+Cor-P vs Cor+BMP-K+Cor+BMP-P				
16 weeks	9/9	0.063	0.011	0.024
Cor-K vs autograft				
16 weeks	6/6	0.002	0.015	0.002
36 weeks	6/6	0.002	0.004	0.002
Cor-K+Cor-P vs autograft				
16 weeks	9/6	0.001	0.003	0.001
Cor+BMP-K vs.autograft				
16 weeks	6/6	0.009	0.240	0.026
36 weeks	6/6	0.004	0.009	0.002
Cor+BMP-K+Cor+BMP-P vs autograft				
16 weeks	9/6	0.066	0.456	0.181

Table 5 Maximal torque capacity (MTC) of the bones. The values are shown as mean±SD. For the description of groups, see Table 1

	Number of unstable bones [a]	MTC (Nm)
Cor-K	6/6	–
Cor-P	0/3	0.10±0.11
Cor+BMP-K	2/6	0.17±0.08
Cor+BMP-P	0/3	0.44±0.57
Autograft	0/6	1.76±0.66

[a] Manually unstable bones were not tested mechanically

Fig. 4A, B Photomicrographs of 6 µm thick sections of (**A**) a coral implant showing fibrous non-union, imaged with a macro lens over a precision desktop illuminator (image width 20 mm), and (**B**) a composite coral implant with BMP showing bone formation on both sides of the fracture line reconstructed after the torsion test, imaged with a microscope using 1× objective (*scale bar:* 1 mm). Masson-Goldner trichrome stain



Resorption of the implant

The mean score for resorption was 1.2 in the group Cor-K, where only 1 implant was resorbed completely and 2 showed no resorption at all. Resorption of the implant was significantly different (Mann-Whitney test, P=0.026) in the group Cor+BMP-K compared to the group Cor-K, 5 of the 6 implants being completely resorbed and 1 almost completely resorbed (mean score

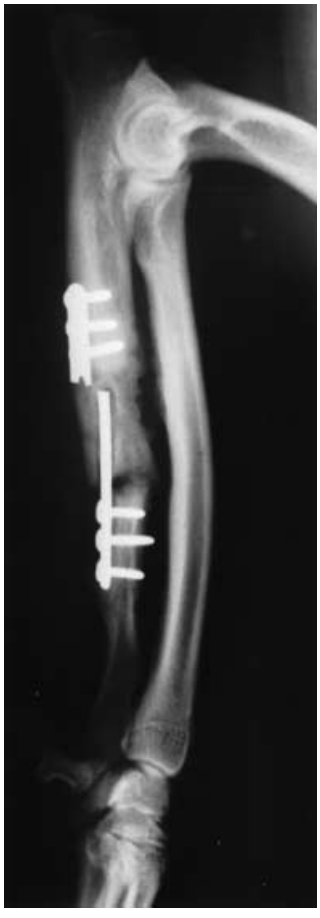


Fig. 3 A radiograph at 16 weeks showing good healing at the proximal end and partial healing at the distal end of a defect treated with a composite implant of coral and BMP, despite breakage of the plate used for fixation

2.8). Resorption was faster in the group Cor+BMP-K, being nearly complete at 12 weeks, compared to a similar outcome at 16–28 weeks in the group Cor-K. In the case of plate fixation, all 3 implants in the group Cor+BMP-P were completely resorbed at 9–12 weeks (resorption score 3.0), while in the group Cor-P, 2 of the 3 implants were also completely resorbed and 1 almost fully resorbed (mean score 2.7), the resorption time being 12–16 weeks.

Mechanical testing

All the bones that were manually stable were tested. Thus, all the bones in the group Cor-K and 2 bones in the group Cor+BMP-K were excluded. All the bones with coral implants with or without BMP broke at the implant area in torsional testing, while the fracture line in the bones with autograft implants occurred outside the implant. The Mann-Whitney test resulted in a significant difference in the mechanical strength between the coral implants with and without BMP (P=0.04). The mechanical strength of the coral implants, even with BMP, was significantly lower than the strength of the autografts (P<0.01). Table 5 shows the mean MTC values of the bones that were tested.

Histology

The bones that showed non-union in radiograms were also seen to have fibrous non-union histologically (Fig. 4a). In the group Cor+BMP-K, there was newly formed bone at the sites where the resorbed implant had been, and bone was bridging the defect in 3 cases. In the group Cor+BMP-P, 1 case showed extensive fibrosis between the bone ends, while 2 cases also showed bone union histologically (Fig. 4b). The two autograft implants where the distal union was not complete showed cartilaginous callus filling the gap.

Discussion

Coral implants have been available for clinical use in cases with a bone defect or unsatisfactory bone union and have been experimentally evaluated in various bone defect models. Guillemin et al. [11] studied the capacity of coral implants to heal canine femoral defects. Both cortical and spongy bone defects were at least partially filled with new bone after 8 weeks, while the implants underwent continuous resorption. In a previous study [10], we compared biocoral and tricalciumphosphate (TCP) in sheep tibial defects, and the conclusion was that coral seemed to be superior to TCP in repairing of segmental defects in weight-bearing limbs.

The capacity of native BMPs to induce bone formation and heal bone defects has been studied in various animal models with favorable results in most cases [9, 13, 21, 23]. However, some authors have also reported failures in bone union [13,16]. The efficacy of recombinant BMP in experimental studies has also been demonstrated [3–5, 18, 29]. In this study, native bovine BMP clearly improved bone healing when evaluated roentgenologically, histologically and mechanically, supporting the earlier findings.

Few previous studies have focused on the combined effect of coral and BMP in bone healing. Gao et al. [9] found a larger amount of newly formed external callus when using a composite implant consisting of coral and native moose BMP in a segmental tibial defect in sheep at 6 weeks, compared to plain coral. However, no mechanical superiority in favor of the composite implant was observed at 16 weeks. Sciadini et al. [23] used the canine radial defect model with bovine-derived BMP with a natural coral carrier. The results showed that all the composite implants with coral and BMP gained union while implants without BMP failed to unite. Coral combined with BMP performed consistently better than the autogenous cancellous bone graft in terms of the extent of bone formation and the strength of the healed defect. The present results were better than those of Gao et al. [9], but not as good as those of Sciadini et al. [23]. The difference of the results in comparison with those of Sciadini et al. [23] may be due to the different autograft materials. Here, the difference in the mechanical environment between the coral implants and the autografts may have impaired the results. However, our results demonstrated the efficacy of BMP in bone induction, although autograft bone was superior to coral implants, even when used with BMP.

The effect of the fixation method has also to be considered. The Kirschner wire used by us does not give rotational stability. Because of this problem we used plate and screw stabilization in 3 dogs, which gives better mechanical stability. The plate we used was too weak and broke in the majority of cases after the first few weeks. However, it may have provided initial stability during the first few weeks, which favors bone union. On the other hand, there are studies where the canine ulnar defect model has been used without any fixation and the bone defects have united with BMP implants [3, 21]. More systematic studies are needed to elucidate the effect of fixation.

Natural coral is a resorbable bone substitute material. The speed of resorption is not optimal, being too slow in some cases, although it has been demonstrated to resorb more quickly than hydroxyapatite [26]. Here, BMP enhanced the quantity and speed of coral resorption. The actions of cellular and interstitial fluids have been suggested as possible agents for coral resorption [6, 7, 11], but the mechanism is still unknown. At any rate, the accelerated resorption may have a favorable effect on the bone healing process.

In conclusion, coral seems to be a biocompatible, resorbing material and thus suitable for use as a bone substitute. BMP seemed to enhance the ability of plain

coral to heal an ulnar bone defect, but this combination was not as good as autologous cortico-cancellous bone with the fixations used.

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