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Authors

Verstraete, Frank JM
Arzi, Boaz
Huey, Daniel J
et al.

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Regenerating Mandibular Bone Using rhBMP-2: Part 2—Treatment of Chronic, Defect Non-Union Fractures

Frank J.M. Verstraete[1], DrMedVet, Diplomate AVDC, ECVS & EVDC, Boaz Arzi[1], DVM, Diplomate AVDC & EVDC, Daniel J. Huey[2], PhD, Derek D. Cissell, VMD, Diplomate ACVR, and Kyriacos A. Athanasiou[2,3], PhD, PE

[1] Department of Surgical and Radiological Sciences, School of Veterinary Medicine, University of California, Davis, California ,[2] Department of Biomedical Engineering, College of Engineering, University of California, Davis, California and [3] Department of Orthopedic Surgery, School of Medicine, University of California, Davis, California

Corresponding Author
Dr. Frank J.M. Verstraete, DrMedVet, Diplomate AVDC, ECVS & EVDC, Department of Surgical and Radiological Sciences, School of Veterinary Medicine, University of California, Davis, One Shield Avenue, Davis, CA 95616.
E-mail: fjverstraete@ucdavis.edu

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Objective: To describe a surgical technique using a regenerative approach and internal fixation for reconstruction of critical size bone defect non-union mandibular fractures.
Study Design: Case series.
Animals: Dogs (n = 6) that had internal fixation of defect non-union mandibular fracture.
Methods: In 5 dogs, the repair was staged and extraction of teeth performed during the initial procedure. After 21–98 days (mean, 27 days) pharyngotomy intubation and temporary maxillomandibular fixation were performed. Using an extraoral approach, a locking titanium miniplate was contoured and secured to the mandible. A compression resistant matrix (CRM) infused with rhBMP-2 was implanted in the defect. The implant was then covered with a soft tissue envelope followed by surgical wound closure.
Results: All dogs healed with intact gingival covering over the mandibular fracture site defect and had immediate return to normal function and correct occlusion. Hard-tissue formation was observed clinically within 2 weeks and solid cortical bone formation within 3 months. CT findings in 1 dog at 3 months postoperatively demonstrated that the newly regenerated mandibular bone had 92% of the bone density and porosity compared to the contralateral side. Long-term follow-up revealed excellent outcome.
Conclusion: Mandibular reconstruction using internal fixation and CRM infused with rhBMP-2 is an excellent solution for the treatment of critical size defect non-union fractures in dogs.

Individual mandibular fractures occasionally fail to heal resulting in non-union, defined as failure of the opposing fracture ends to unite and to ossify.[1] The amount of healing that occurs varies from fibrous connective tissue, cartilaginous bridge that does not mineralize, or absolute lack of bridging.[1] According to the Weber–Cěch classification, a defect non-union occurs when a section of the bone is lost during a trauma, because of sequestration or after surgery.[1,2] The resulting gap between the remaining viable bone ends is too great to be bridged without surgical intervention.[1,2] Common predisposing causes for non-union include comminution, infection, ischemia, hyperemia, excessive manipulation and hardware placement, periosteal stripping too early or excessive mobility and imperfect reduction.[1] Radiologically, features such as absence of callus, evidence of a fracture gap, sclerosis of the fractures end, and displacement are typically present.

A common result of defect non-union fractures is malocclusion because of mandibular drift.[3–6] Malocclusion may result in difficulty in eating and drinking, prehension and pain of the contralateral temporomandibular joint (TMJ).[3–5,7] Therefore, the primary objective for repair of mandibular fractures, including non-union, is a quick return to normal function and restoration of normal occlusion.[8] However, while mandibular reconstruction represents the ideal solution the aspects of this technique including choice of graft material to bridge the defect and matching anatomic geometry make this approach challenging.[9,10] Autologous bone grafts and bone graft substitutes are examples of the techniques available to address the problem.[6,9,11,12] However, these are still far from ideal because of donor site morbidity, especially in small dogs.[9,13,14]

Work performed in the Department of Surgical and Radiological Sciences, School of Veterinary Medicine, and Department of Biomedical Engineering, College of Engineering, University of California, Davis.

Bone morphogenetic proteins (BMPs) are multifunctional growth factors within the transforming growth factor β (TGF-β) super family that were identified by Urist based on their ability to initiate ectopic bone formation.[15–18] Later, Reddi proposed that BMPs are responsible for the initiation of a cascade of developmental events, in which progenitor cells are induced to differentiate into bone cells thus resulting in new bone formation.[19,20] This unique feature of BMP has allowed for their successful use as therapeutic agents for bone repair.[18] Indeed, much work has been done with the clinical use of recombinant human BMPs (rhBMPs) in spinal fusion, fracture healing, and engineering of dental tissues.[21,22] Currently, rhBMP-2 and rhBMP-7 delivered via adsorption onto collagen matrices are FDA approved for spinal fusion.[21,23–25]

Our purpose is to report our experience gained from applying a collagen and calcium ceramic matrix impregnated with rhBMP-2 to effect bone regeneration in 6 dogs undergoing reconstruction for treatment of mandibular defect non-union fractures.

MATERIALS AND METHODS

Case Recruitment and Treatment Planning

Dogs with defect non-union fractures were evaluated for the degree of malocclusion and functionality. All 6 dogs had marked malocclusion. The date of the initial fracture was unknown because all dogs were adopted from a shelter without further history. In 2 dogs the duration of the non-union was known to be ~2 and 5 years, respectively. Full-mouth dental radiographs were obtained in all dogs and computed tomography (CT) with tridimensional reconstruction was performed in 5 dogs (Figs 1 and 2).

Staging, Extractions and Debridement

In 5 dogs, the repair was staged. During the initial procedure periodontal treatment was performed and in 4 dogs the teeth (n = 2–3; mean 2.5) in or adjacent to the non-union fracture site were extracted. The teeth involved included premolar tooth P2 (n = 1), P3 (1), P4 (4), and molar tooth M1 (3), and M2 (1). In 1 dog there were no teeth related to the non-union but extensive periodontal treatment and multiple extractions elsewhere were performed. One dog was edentulous and the procedure was completed in a single session. The non-union site was debrided in all dogs, which included removal of an intraosseous wire in 1 dog (Fig 2).

CRM and rhBMP-2 Preparation

CRM (collagen sponge with embedded granules of hydroxyapatite [HA] and tricalcium phosphate [TCP]; MasterGraft Matrix® Medtronic, Memphis, TN) and rhBMP-2 (Pfizer, Cambridge, MA) were used in this study. The volume of the defect was measured in 3 dimensions and a sufficient amount of CRM (i.e., to provide a half to three-quarters of the mandibular height and a

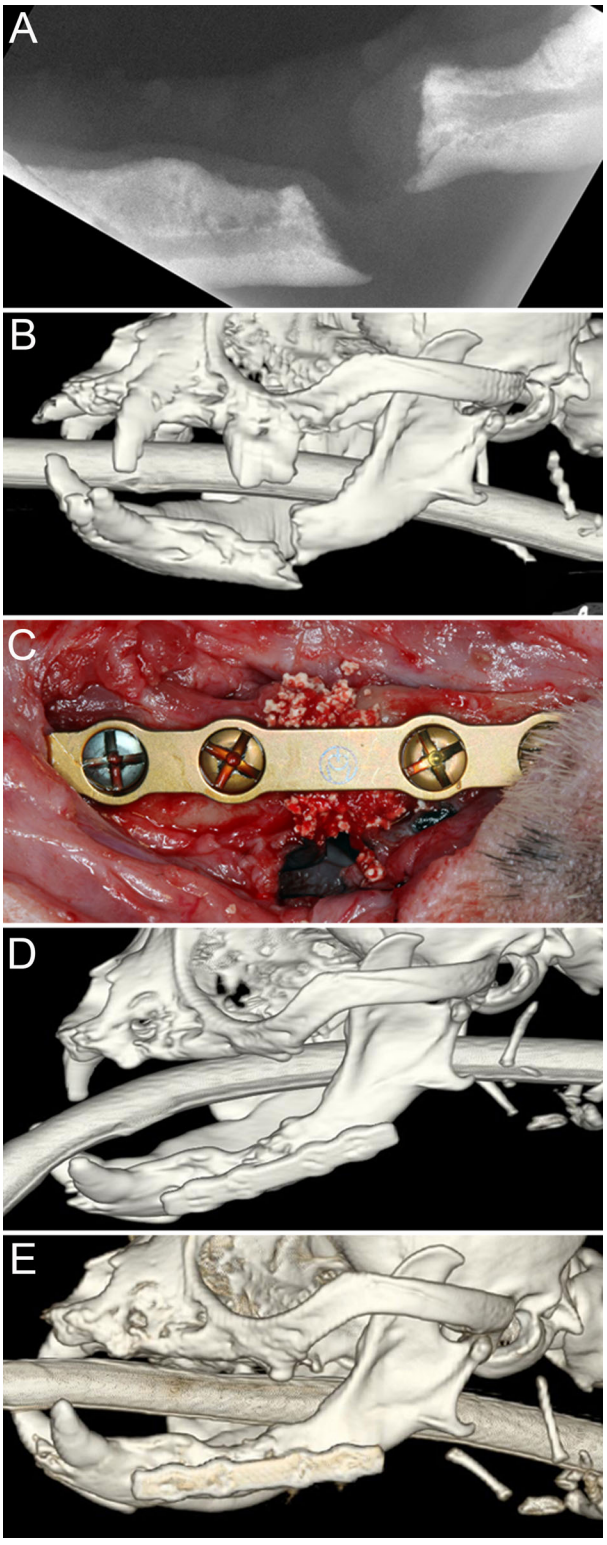

Figure 1 Defect non-union mandibular fracture of 2-year duration in a 4.3 kg, 7.3-year-old dog. (A) Dental radiograph of the affected mandible; (B) tridimensional CT reconstruction; (C) 2-mm locking plate fixation with rhBMP-2 in a CRM; (D) 3-month; and (E) 14-month follow-up tridimensional CT reconstructions showing solid and stable new-bone formation.

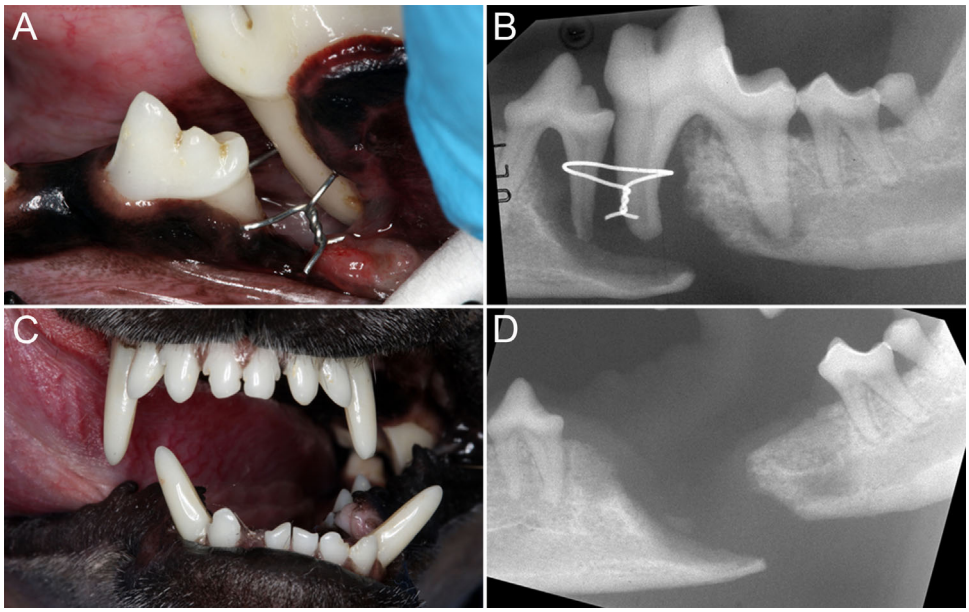

Figure 2 Defect non-union mandibular fracture with teeth (and an orthopedic wire) in the fracture line, of unknown duration in a 6 kg, 3-year-old dog. (A) Oral view showing the defect, exposed roots and orthopedic wire; (B) dental radiograph of the affected mandible; (C) malocclusion visible at the 3 weeks recheck after dental extractions and debridement; (D) dental radiograph at the 3 weeks recheck showing the critically sized defect.

length 2 mm greater than defect span) was measured. Fifteen minutes before implantation, the CRM was infiltrated with a 0.5 mg/mL rhBMP-2 at a volume corresponding to 50% of the volume of the prepared CRM. For example, for a CRM that was 2 cm in length, 0.5 cm mandibular width and 1 cm mandibular height ($2 \times 0.5 \times 1$), the total defect volume is 1 cm^3; thus, 0.5 mL of the rhBMP-2 solution was used.

Surgical Technique

In all dogs, ampicillin (20 mg/kg intravenously [IV]) was administered at the time of induction. Dogs were intubated via pharyngotomy.[26] The mandibles and maxillas were brought into the desired closed-mouth occlusion and the mandibular and maxillary canine teeth wired together using 28 g wire (Fig 3).

Using an extraoral approach, a single locking titanium 2-mm 4 or 6-hole miniplate (Synthes® Maxillofacial, Paoli, PA) was adapted to the desired anatomic contour of the mandible in a ventrolateral position while avoiding tooth root damage. No tooth roots were damaged by the screws. The fracture edges were debrided to remove sclerotic bone and attached soft tissues using a surgical hand-piece designed for major oral surgery (INTRAsurge 300, KaVo America Corp., Lake Zurich, IL) combined with an ostectomy bur (Lindemann bur, Hu-Friedy, Chicago, IL) or with rongeurs. The plate was then secured to the bone with 2–3 locking titanium screws in each segment of the fracture. The surgical area was copiously irrigated with sterile saline solution.

Measurements of the defect were used to guide the preparation of the appropriate size CRM, which was soaked in a solution of rhBMP-2 as described previously. The soaked

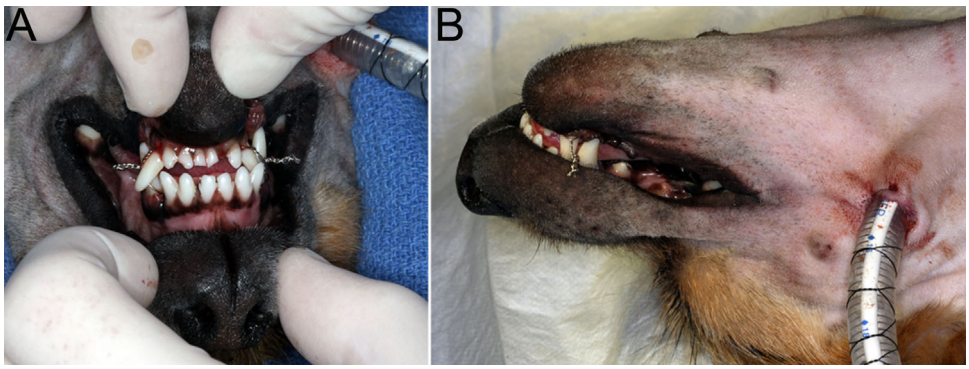

Figure 3 (A) Rostral and (B) lateral images of the dog in Fig 2 showing the occlusion maintained with maxillomandibular fixation and pharyngotomy intubation. A small oral fenestration is visible, which was subsequently sutured intraorally.

CRM was then implanted in the defect and the surrounding soft tissues were sutured around the plate and sponge to provide a soft tissue envelope. Care was taken to avoid fenestration into the oral cavity but when it occurred the oral mucosa was sutured. The subcutaneous tissues and skin were closed in layers.

Dogs were fed soft food for 2 weeks after surgery and were administered amoxicillin/clavulanic acid 20 mg/kg orally twice daily for 1–2 weeks postoperatively. Postoperative pain management typically consisted of a combination of a non-steroidal anti-inflammatory drug and an opioid analgesic.

Diagnostic Imaging

Radiographs of the mandible were obtained immediately postoperatively and at 2–4 weeks, 8 weeks, and 12 weeks after surgery. Radiographs were obtained at later time points if indicated. Transverse, 0.625-mm, collimated X-ray CT images (LightSpeed 16; GE Healthcare, Milwaukee, WI; kVp = 120 and auto-mA) of the skull were acquired in 1 dog 3 and 14 months after surgery. All images were reconstructed using a bone filter. A CT calibration phantom containing 5 reference rods of known density (Mindworks Software, Inc.; San Francisco, CA) was included in the field of view during CT image acquisition. All radiographs and CT images were evaluated qualitatively by a board-certified veterinary radiologist (DDC) using DICOM image viewing software (OsiriX v. 5.0.2, Geneva, Switzerland). CT images were also evaluated quantitatively using data analysis software (MATLAB R2011a; Mathworks®, Natick, MA). For quantitative measurements, 4 transverse CT images were selected at regular intervals along the length of the mandibular repair. The porosity and radiographic mineral density were measured for the native mandible and mandibular repair tissue using freeform regions of interest that included the entire cross section of the mandible, but excluded the tooth roots or metal surgical implants. The 4 measurements were averaged to reduce error associated with measurement and image-to-image variability.

RESULTS

All dogs had good physical condition and results of hematologic, serum biochemical profile, and urinalysis were generally considered normal. In 6 small breed dogs, aged 2–11 years (mean, 7.3 years), and weighing 3.4–6 kg (mean, 4.8 kg) a single 2-mm locking miniplate (Synthes® Maxillofacial) was used to stabilize defect non-union fractures of 5–18 mm (mean, 9.2 mm). Follow-up was 6–21 months (mean, 12 months).

Clinical Evaluation

All dogs had appropriate occlusion immediately postoperatively and throughout follow-up. Besides restriction of heavy chewing (e.g., no rawhide chewing or rough games) for 3 months, all dogs returned to normal activity after surgery. At 2 weeks, hard tissue spanning the entire defect site was palpable and covered by intact gingiva. There was no noticeable oozing from the intraoral and extraoral incision sites during the reported follow-up. At 4 weeks, the defect felt completely solid and no abnormalities were noticed. During the rest of the follow-up period there was no recurrence of the fractures and functional occlusion.

Imaging Findings

Immediate postoperative radiographs were available for all dogs and follow-up radiographs available for 5 dogs (representative radiographs: Figs 1, 2, and 4). One dog had a slight decrease in opacity of the implant material at 2 weeks. All dogs with recheck radiographs had increased opacity of the repair site with smoothly margined new bone formation bridging between the implant material and native mandible on radiographs obtained at 4 weeks or later (Fig 4). The margins of the repair site became increasingly smooth and the transition between implant material and native mandible became progressively indistinct over time. For 1 dog with radiographs

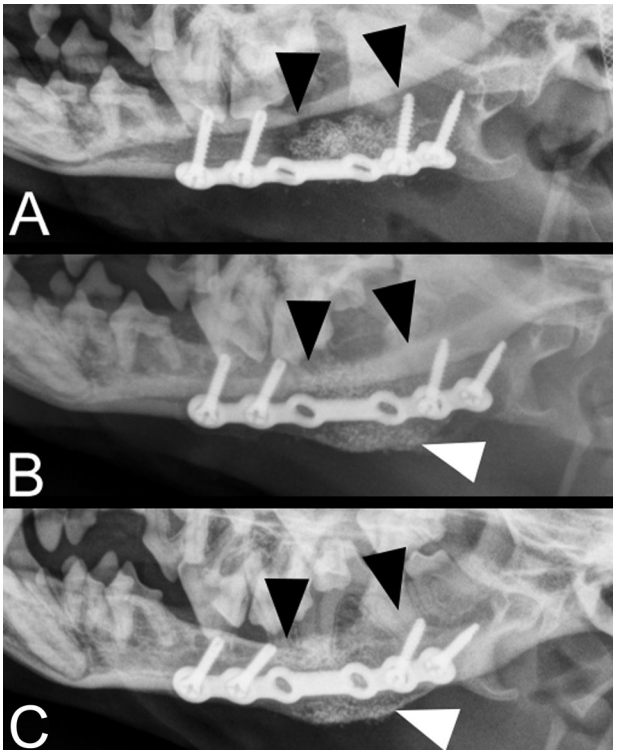

Figure 4 Radiographs of a defect non-union mandibular fracture, of unknown duration in a 5.2 kg, 2.0-year-old dog. (A) Immediately postoperatively; (B) 4 weeks; and (C) 8 weeks after surgery. The black arrowheads indicate the approximate borders of the implanted rhBMP-2 CRM scaffold. Smoothly margined new bone formation is evident at the ventral aspect of the mandibular repair site (white arrowheads) and the gap between the implant material and native mandible is indistinct at 4 and 8 weeks after surgery.

obtained at 7 months, focal irregularity persisted at the dorsal margin of the mandibular repair site, but the implant material otherwise appeared remodeled and well integrated with native mandible.

On postoperative CT images obtained in a single dog, there was radiographic evidence of new bone formation and integration of the implant material with the native mandible at 3 and 14 months (Fig 5). The porosity of the mandibular repair site was similar to native mandible at 3 and 14 months (26.8% and 27.4% porosity at 14 months for native and repair sites, respectively). The radiographic mineral density of the mandibular repair site measured 0.90 times that of the native mandible at 3 months and measured 0.96 times the density of the native mandible at 14 months.

DISCUSSION

We are unaware of other reports of the use of rhBMP-2 delivered via adsorption into a CRM for regenerating bone across chronic defect non-union fractures in dogs. Importantly, our findings emphasize the benefits of using rhBMP-2 delivered in CRM for their profound and predictable regenerative capacity in mandibular reconstruction in dogs.

Overall, using this combined surgical and regenerative strategy in small dogs was able to rapidly return chronically afflicted dogs to normal occlusion and function. This was because the surgical approach allowed restoration of normal anatomy and occlusion, and bone regeneration restored functional biomechanics. Palpable bone quickly formed using the CRM infused with the appropriate dosage of rhBMP-2. By 3 and 14 months, this tissue radiographically approximated the density of and appeared integrated to native bone. Histologically, previous reports confirmed that CRM infused with rhBMP-2 resulted in well-mineralized trabecular bone reflective of healthy bone turnover and remodeling.[5,27,28]

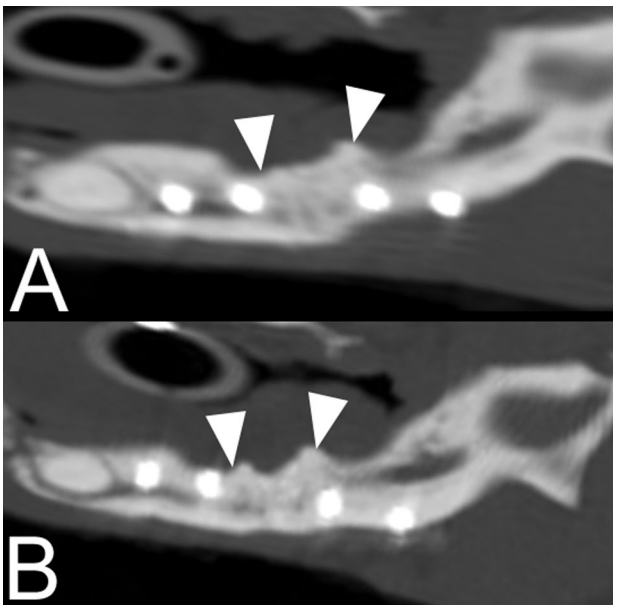

Figure 5 Sagittal reconstructed CT images of the left mandible of the dog in Fig 1 (A) 3 months and (B) 14 months after surgery. White arrowheads indicate the approximate borders of the original implant material. No gap is visible at the repair site and the implant material appears to be integrated with native mandible.

Importantly, our findings agree with several human case reports demonstrating that successful reconstruction of critical size mandibular defects can be achieved without the use of an autograft or other form of bone grafts.[9,10,29] In experimental studies that used the same regenerative system (i.e., CRM and rhBMP-2), successful spinal fusion and mandibular reconstruction in non-human primates, dogs, and rabbits because of robust formation of bone approximating native tissue was observed.[25,27,30] However, our report is unique in that we successfully treated chronic mandibular defects that are pathophysiologically different from fresh defects.[31]

The therapeutic outcome after use of rhBMP-2 critically depends on the delivery vehicle, quantity, concentration, and time of application.[32,33] Use of rhBMP-2 without a carrier is contraindicated and the selection of the matrix used for delivery must be carefully considered.[34] In our study and others, CRM proved to be appropriate for the delivery and release of rhBMP-2 at the defect site.[5,9,25,29] In a rat critical bone defect model where rhBMP-2 was used, the degree of bone formation was dose dependent[25,28,30,35]; however, increasing rhBMP-2 dose beyond a certain threshold concentration does not improve bone quality, and may promote lower quality bone and invoke a detrimental inflammatory response.[28] We used a uniform dose of 0.5 mg/mL with a 50% soak volume and bone approximating native geometry and density formed within the critical size defect and was well integrated to adjacent native tissue. However, in cases where a higher dosage of rhBMP-2 was applied there was initial excessive bone formation but this resolved within several months.[7,27,36] Although we did not evaluate a series of concentrations, we conclude that the dose generally used in this study is clinically appropriate.

Not only is the dose of rhBMP-2 critical to obtain bone formation, there must be appropriate cells and these cells must have the ability to respond to the cytokine. Thus, the success of rhBMP-2 application in our approach was because of the presence of appropriate stem cells in the local environment and their ability to differentiate into bone forming cells.[9] Although, it is accepted that with increasing age the number of stem cells available decrease,[27,37] the osteogenic capabilities of rhBMP-2 are not negatively affected by increasing age.[27] In agreement with this, we observed excellent clinical outcome suggesting that the presence and osteogenic ability of the resident stem cells in older age dogs is sufficient.

The procedure was staged to resolve infection related to periodontitis, remove teeth in the fracture line or affected by severe periodontitis, and remove previous failed implants and granulation tissue in the defect non-union. Our rationale was to provide a more favorable environment for a subsequent regenerative procedure. Bacterial culture was not performed as the tissues appeared healthy at the time of the second surgery.

To avoid subsequent surgery we recommend that a single 2-mm titanium locking miniplate, placed in the mid-part of the mandibular body be used. This approach avoids iatrogenic damage to the teeth roots, is sufficient to buttress the defect, does not result in plate failure, and avoids plate exposure through the mucosa.

Despite the regenerative capacity of skeletal tissue, the biological processes sometimes fail and fractures may heal in non-union.[31] Using rhBMP-2 with CRM has been demonstrated to be crucial in the initiation of bone healing cascade and formation of new bone.[15,16,29,31,38] The combined surgical and regenerative methodology we report achieved predictable, timely reconstruction of defect non-union fractures in small breed, older dogs. Use of rhBMP-2 should not be taken lightly as this is a very potent molecule that has wide-ranging functions and versatility and is dose dependent.[28,39] Finally, incorporating regenerative technology into the surgical arena of managing defect non-union fractures is providing exciting possibilities that eliminate or minimize the morbidity associated with bone grafting and allow for a quick return to normal function.

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DISCLOSURE

The authors report no financial or other conflicts related to this report.

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