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Authors

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

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Regenerating Mandibular Bone Using rhBMP-2: Part 1 – Immediate Reconstruction of Segmental Mandibulectomies

Boaz Arzi¹, DVM Diplomate AVDC & EVDC,

Frank J.M. Verstraete¹, DrMedVet, MMedVet, Diplomate AVDC, ECVS & EVDC, Daniel J. Huey², PhD, Derek D. Cissell^{1,2}, VMD, Diplomate ACVR, and Kyriacos A. Athanasiou^{2,3}, PhD, PE

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

Corresponding Author

Dr. Frank J.M. Verstraete, DrMedVet, MMedVet, 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 immediate reconstruction of critical size bone defects after segmental mandibulectomy in dogs.

Study Design: Prospective case series.

Animals: Dogs (n = 4) that had reconstruction after segmental mandibulectomy for treatment of malignant or benign tumors.

Methods: Using a combination of extraoral and intraoral approaches, a locking titanium plate was contoured to match the native mandible. After segmental mandibulectomy, the plate was secured and a compression resistant matrix (CRM) infused with rhBMP-2, implanted in the defect. The implant was then covered with a soft tissue envelope followed by intraoral and extraoral closure.

Results: All dogs that had mandibular reconstruction healed with intact gingival covering over the mandibular defect and had immediate return to normal function and occlusion. Mineralized tissue formation was observed clinically within 2 weeks and solid cortical bone formation within 3 months. CT findings at 3 months showed that the newly regenerated mandibular bone had ~50% of the bone density and porosity compared to the contralateral side. No significant complications occurred.

Conclusion: Mandibular reconstruction using internal fixation and CRM infused with rhBMP-2 is an excellent solution for immediate reconstruction of segmental mandibulectomy defects in dogs.

A common end result of critical size mandibular bone defects (i.e., a bony defect that will not heal by bone formation during the lifetime of the animal) after segmental mandibulectomy is malocclusion because of mandibular drift.¹⁻⁷ Malocclusion can result in difficulty in eating and drinking, prehension and pain of the contralateral temporomandibular joint (TMJ).^{1-3,8} Whereas mandibular reconstruction represents the ideal solution, several aspects of this technique including the choice of graft material and matching anatomic geometry make this approach challenging.^{9,10} Autologous bone grafts, bone graft substitutes, microvascular tissue transfer, and distraction osteogenesis are examples of techniques available to address the problem.^{4,9,11,12} However, these are still far from ideal because of donor site morbidity, scarce tissue availability, and limitation in graft size and contour.^{9,13,14}

Our group has investigated procedures to prevent mandibular drift after mandibulectomy.^{1,2} First, mandibular rim excision with preservation of the ventral border is a sound technique for small odontogenic or malignant tumors in medium and large

dogs.¹ However, this technique is not recommended for more invasive tumors or in small dogs. Second, elastic training is a viable option for preventing mandibular drift but requires good client compliance and it only prevents mandibular drift in approximately half of the dogs.² Thus when segmental mandibulectomy is required, the ideal treatment should be anatomically correct reconstruction of the mandible, potentially through bone regeneration, to allow appropriate biomechanics and thus functional pain-free occlusion.^{9,15}

It has been over 40 years since Urist's pioneering work discovering the family of active compounds responsible for bone regeneration, the bone morphogenetic proteins (BMPs).^{16,17} Motivated by this, Sampath and Reddi created a bioassay for BMP based on the formation of ectopic bone.^{18,19} Reddi proposed that BMPs are responsible for the initiation cascade of developmental events, in which progenitor cells are induced to differentiate into bone cells thus resulting in new bone formation.^{20,21} Much work followed with the clinical use of recombinant human BMPs (rhBMPs) in the field of spinal fusion, fracture healing, and engineering of dental tissues.^{22,23} This work resulted in FDA approval of 2 spinal fusion products consisting of either rhBMP-2 or rhBMP-7 delivered via absorption onto collagen matrices.^{18,22,24,25}

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

The multifunctional growth factors of the BMP family comprise over 20 distinct ligands and play an important role not only in bone formation and remodeling but also in development and regeneration after tissue damage.^{26,27} Moreover, BMPs induce a plethora of different cellular effects ranging from stem cell maintenance, migration, differentiation, proliferation, and apoptosis.²⁷ Because these proteins also play important roles in various other processes unrelated to bone including iron and energy metabolism, and adipogenesis, Reddi proposed naming this family of growth factors body morphogenetic proteins.^{26,28,29}

Although mandibular reconstruction using titanium locking plates and rhBMP-2 delivered in a scaffold has been described in isolated case reports in people and animals, we report this technique performed prospectively with additional considerations and refinement.^{3,8-10} Specifically, we report experience gained from applying a collagen and calcium compression resistant matrix (CRM) impregnated with rhBMP-2 to effect bone regeneration in 4 dogs undergoing reconstruction after segmental mandibulectomy.

MATERIALS AND METHODS

Case Recruitment

Dogs requiring segmental mandibulectomy for odontogenic or malignant tumors were recruited for this study. A signed informed consent was obtained from the clients. All dogs had preoperative minimal data base (e.g., complete blood count, serum biochemical profile, and urinalysis). Dogs were staged by means of abdominal ultrasonography and thoracic radiography or computed tomography (CT).³⁰ Mandibular lymph nodes were fine-needle aspirated and submitted for cytologic analysis. Dogs were evaluated at a regular intervals of 2, 4, 8, and 12 weeks postoperatively and then every 6 months for the duration of the reported follow-up period.

CRM and rhBMP-2 Preparation

The CRM (collagen sponge with embedded granules of hydroxyapatite [HA] and tricalcium phosphate [TCP]; Master-Graft 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 length 2 mm greater than the defect span) was measured. Fifteen minutes before implantation, the CRM was infiltrated with 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 5 cm in length, 1 cm mandibular width and 1.5 cm mandibular height ($5 \times 1 \times 1.5 \text{ cm}^3$), the total defect volume was 7.5 cm^3 ; thus, 3.75 mL of rhBMP-2 solution was used.

Surgical Technique

Detailed description of preoperative oral care, mandibulectomy techniques and principles of internal fixation are

beyond the scope of this report and can be viewed elsewhere.^{1,3,4,8,31,32} Briefly and specifically, the mandible was accessed by extra and intraoral approaches.³³ After measurements and marking of the resection area (Fig 1A), a single titanium locking plate (3.0 mm, Synthes[®] Maxillofacial, Paoli, PA) was contoured before the amputation, capturing the normal anatomic contour of the ventrolateral aspect of the mandible. The plate was secured to the bone with appropriate size titanium locking screws (Fig 1B). The plate and screws were removed and the segmental mandibulectomy was started extraorally and completed intraorally. Then, resection of the mandible ensued with appropriate surgical margins and intraoral closure (Fig 1C), the plate was repositioned and secured to the mandible by the extraoral approach. The surgical site was copiously irrigated with sterile saline solution as after CRM implantation, irrigation is no longer possible. The soaked CRM was then implanted in the defect to fit snugly and secured circumferentially with poliglecaprone-25 suture to prevent migration after implantation (Fig 1D). A new surgical pack and new surgical gloves were used for closure. The surrounding soft tissues were sutured around the plate and CRM to provide a soft tissue envelope, and the subcutaneous tissues and skin closed in layers.

Dogs were fed soft food for 2 weeks after surgery and were administered ampicillin (20 mg/kg intravenously [IV]) preoperatively and amoxicillin/clavulanic acid (20 mg/kg orally twice daily for 2 weeks) postoperatively. Analgesia was achieved by administration of opioids and non-steroidal anti-inflammatory medications for 7–14 days.

Diagnostic Imaging

Radiographs of the mandibles were obtained using a digital radiography system (RapidStudy EDR6, Eklin Medical Systems, Sunnyvale, CA) immediately postoperatively and at 2, 4, 8, and 12 weeks after surgery. Radiographs were obtained at longer time points (e.g., 5–6 months) if indicated.

Transverse, 0.625-mm, collimated CT images (Light-Speed 16; GE Healthcare, Milwaukee, WI; kVp = 120 and auto-mA) of the mandibles were obtained for 2 dogs 3 months after surgery. 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 image acquisition.

CT images were evaluated qualitatively and quantitatively using DICOM viewing software (OsiriX v. 4.1.2 32-bit; Geneva, Switzerland) and 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 Hounsfield units (HU), bone density, and porosity were measured for the mandibular repair tissue using freeform regions of interest that excluded the tooth roots and mandibular canal. The 4 measurements were averaged to reduce error associated with measurement and image-to-image variability.

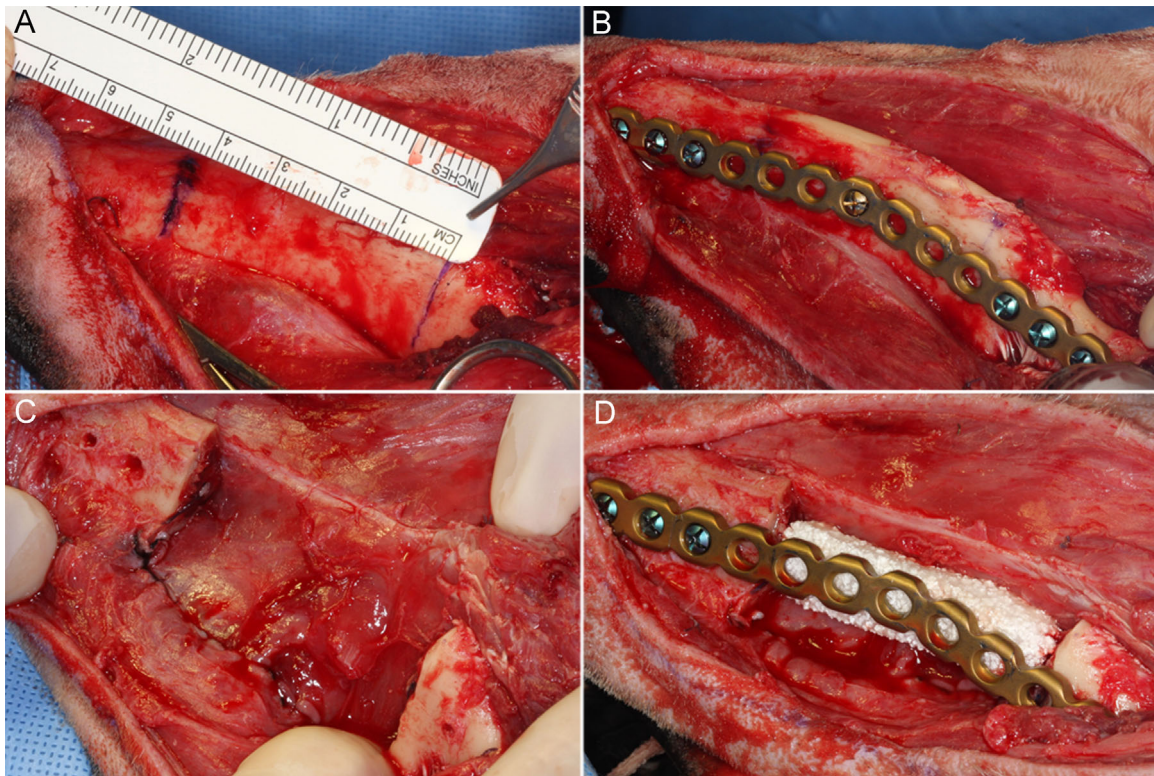


Figure 1 (A) Intraoperative view demonstrating the extraoral approach and measurements and marking of the resection area; (B) the plate is secured to the bone with appropriate size titanium locking screws and then removed; (C) after segmental mandibulectomy; (D) the plate is returned and secured and the soaked CRM is implanted in the defect.

RESULTS

All dogs had good physical condition and results of hematologic, serum biochemical analysis, and urinalysis were generally considered normal. One dog had preexisting lymphangiectasia and associated mild hypoproteinemia. Thoracic radiographs and abdominal ultrasonography performed during tumor staging revealed no abnormalities. No surgical complications occurred and no neoplastic cells were identified in the surgical margins of the submitted specimens.

Mandibulectomy

Four dogs, aged 8–9 years (mean, 8.8 years) weighing 25–37 kg (mean, 29 kg) had segmental mandibulectomy. Defect size was 42–60 mm (mean, 50.5 mm) for the removal of squamous cell carcinoma ($n = 1$) and canine acanthomatous ameloblastoma ($n = 3$). Follow-up was 15–22 months (mean, 19 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 play) 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. Mild oozing from the intraoral incision site was noticeable at 2 weeks in all dogs but completely resolved by the 4th week. One dog had a small cystic lesion in the gingiva that spontaneously resolved after 4 weeks. At 4 weeks, the defect felt completely solid and no abnormalities were noticed. At 2 and 3 months, there was no recurrence of the tumors or fractures affecting the mandibles. For the remaining follow-up period, no abnormalities were noticed and no plate exposure through the mucosa or exuberant bone reactions were noted. Furthermore, all owners reported that the dogs had an excellent quality of life.

Radiological Evaluation

Radiographic opacity of the regenerated mandible increased from postoperative radiographs to 4 weeks after surgery. At 4 weeks, the margins of the implanted scaffold became smoother and had evidence of new bone connecting the implant to the adjacent mandible. At 8 weeks, the implant material continued to increase in opacity and formed a mineralized union with the mandible. One dog had a well-defined, rounded radiolucency in part of the implant material on radiographs at 4 weeks, followed by progressive increase in opacity and formation of normal-appearing cortical bone along

two-thirds of the dorsal margin of the previous defect by 24 weeks. No radiographic evidence of complications related to the bone plate and screws were observed (Fig 2).

On CT images, there was radiographic evidence of new bone formation with complete integration of the implant material with the native mandible (Fig 3). Density and porosity of the repair tissue and contralateral mandible varied widely between dogs. For the 2 dogs examined, the repair tissues achieved 46–54% of the density of the contralateral mandible (3 months after surgery). Moreover, in 1 dog the regenerated bone had similar to slightly greater porosity (1.1 times) compared to the contralateral mandible and in the 2nd dog, the porosity of the repair tissue was much less (0.4 times) than that of the contralateral mandible.

DISCUSSION

We report the use of rhBMP-2 delivered via adsorption into a CRM for regenerating bone across large critical size mandibular defects in 4 dogs. This combined surgical and regenerative strategy resulted in a rapid return to normal function. This was because the surgical approach allowed the correct reconstruction of normal anatomy and occlusion, and bone regeneration restored biomechanical function. Palpable bone quickly formed using CRM infused with an appropriate dosage of rhBMP-2. By 3 months, this tissue radiographically approximated the density of native bone and appeared well-integrated. Histologically, previous reports confirmed that CRM infused with rhBMP-2 results in well-mineralized trabecular bone reflective of healthy bone turnover and remodeling.^{3,34,35}

Our outcomes reinforce findings of several human case reports demonstrating that successful reconstruction of critical size mandibular defects can be achieved without the use of autograft or other form of bone grafts.^{9,10,15} In experimental studies using 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.^{25,34,36}

The therapeutic outcome after use of rhBMP-2 critically depends on the delivery vehicle, quantity, concentration and time of application.^{37,38} Use of rhBMP-2 without a carrier is contraindicated and selection of the matrix used for delivery must be carefully considered.³⁹ In this study and others, CRM proved to be appropriate for the delivery and release of rhBMP-2 at the defect site.^{3,9,15,25} With regards to the concentration, a study that evaluated the application of rhBMP-2 in a rat critical bone defect model found that the degree of bone formation is dose dependent.^{25,35,36,40} However, increasing the dose of rhBMP-2 beyond a certain threshold concentration does not improve bone quality, and may promote lower quality bone and invoke a detrimental inflammatory response.³⁵ 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 dogs where a higher dosage of rhBMP-2

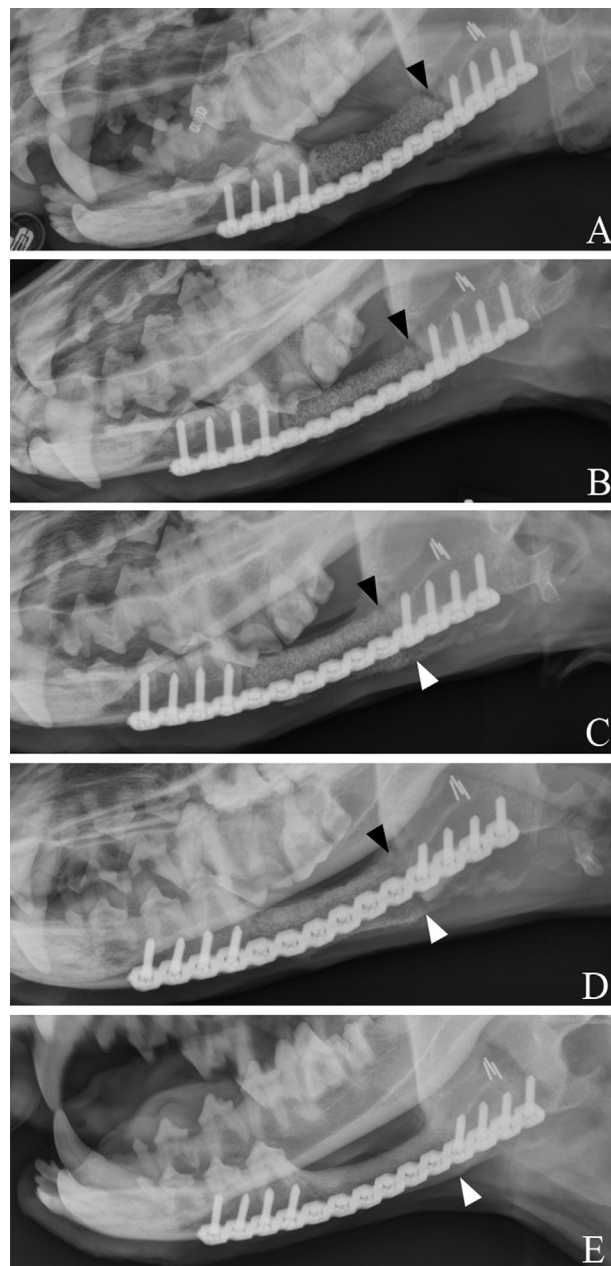


Figure 2 Radiographs of the reconstructed mandible. (A) Immediately postoperatively; (B) 2 weeks; (C) 4 weeks; (D) 8 weeks; and (E) 9 months after surgery. Note the progressive increase in opacity and smoother margins of the implant material from postoperative to 9 months. There is progressive narrowing and opacification of the gap between the caudal aspect of the implant material and native mandible (black arrowheads) and formation of smooth, bridging, mineral opacity repair tissue at the ventral aspect of the junction between the implant and native mandible (white arrowheads). Nine months after surgery, no gap is visible between the implant and native mandible and dense bone resembling normal cortex spans the entire dorsal aspect of the previous osteotomy site.

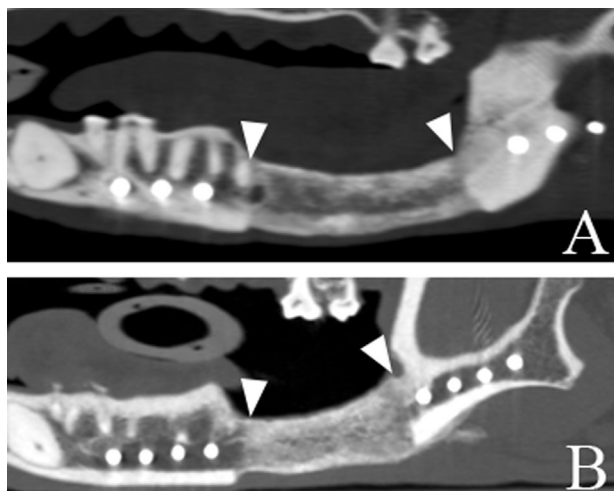


Figure 3 Sagittal reconstructed CT images of the reconstructed mandible 3 months after reconstruction of segmental mandibulectomy in 2 dogs. The approximate borders of the native mandibulectomy are indicated by the white arrowheads. Note the evidence of new bone formation with complete integration with the native mandible.

was applied, there was initial excessive bone formation but this resolved within several months.^{8,32,34} 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 quantity of stem cells available decrease,^{34,41} the osteogenic capabilities of rhBMP-2 are not negatively affected by increasing age.³⁴ In agreement with this, we observed excellent clinical outcome suggesting that the presence and osteogenic ability of the resident stem cells in middle to older age dogs is sufficient.

In 1 dog, we observed a radiographic variation in remodeling in which a rounded, radiolucent bone void was observed, but resolved within 5 months. One study histologically confirmed similar appearing voids to be fatty marrow instead of normal trabecular bone structure.³⁵ This phenomenon could be explained by the fact that at the molecular level, BMP-2 can induce adipogenesis in addition to, or instead of, osteogenesis through activation of transcription factor peroxisome proliferator-activated receptor gamma (PPAR γ), a key regulator of adipocyte commitment.^{29,35,35,42–45} PPAR γ activation leads bone marrow stem cells to differentiate to adipocytes rather than osteocytes and once this occurs, the osteogenic program is suppressed.^{35,46,47}

In all dogs we observed mild oozing from the oral incision site, possibly because of underlying mild inflammation at 2 weeks. This completely resolved by the 4-week recheck examination. Short term BMP-induced inflammation is commonly reported beginning on the 3rd postoperative day,

peaks at 1 week, and typically resolves by 2–3 weeks.^{35,48,49} This response is expected as rhBMP-2 is known to be chemotactic for inflammatory cells including mono- and polymorphonuclear cells and osteoclasts-like cells.^{35,50} We conclude that the general dose and method of application of the rhBMP-2 in our study, although it resulted initially in minimal inflammation and mild oozing, is clinically appropriate because this resolved spontaneously by the 4th week.

Earlier reports described plate exposure through the mucosa.^{3,8} In an attempt to re-establish the alveolar margin, these cases used more than one plate to buttress the defect. Plate exposure on the dorsal aspect was resolved by plate removal and did not negatively affect the long-term excellent outcome.^{3,8} However, using a single larger plate (e.g., 3.0 mm), this complication did not occur. To avoid this complication we recommend using a single 3-mm titanium locking plate, placed on the ventrolateral aspect of the mandibular border. 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, because the overlying soft tissues are not subject to mastication. Therefore, implant removal was unnecessary and would likely be difficult and traumatic, given the osteointegration of titanium plate and screws.

In this study, immediate reconstruction was performed, given the likelihood of achieving tumor-free surgical margins, based on preoperative planning. For more extensive tumors, it may be prudent to stage the procedure because use of rhBMP-2 in a tumor-laden site would be contraindicated. The pre-contoured locking plate could maintain the occlusion until the tumor-free margins are histologically confirmed. However, this would require a second re-entry surgery to place the CRM with rhBMP-2.

We concluded that this combined surgical and regenerative methodology achieved predictable, timely reconstruction of critical size bone defects using a CRM with rhBMP-2. 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.^{26,35} Finally, incorporating regenerative technology into veterinary oral surgery provides 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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