Use of 3D-Printed β-TCP synthetic bone graft combined with rhBMP2 to treat a severe radial atrophic non-union in a Yorkshire Terrier

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Abstract

Objective: To describe a novel surgical approach to treat a critical-sized bone defect due to severe, radial atrophic non-union in a miniature dog.

Study design: Case report.


Methods: Computed tomographic (CT) images of the radius were imported to create for 3-D printing of a custom-designed synthetic β-TCP scaffold. The radius was exposed, and the β-TCP scaffold was press-fitted in the bone gap underneath the plate. RhBMP-2 collagen sponges were squeezed to soak the scaffold with growth factor and then placed on both sides of the synthetic graft. Two additional cortical screws were also placed prior to routine closure of the surgical site.

Results: Radiographic examination was consistent with complete healing of the radius defect four months after surgery. The bone plate was removed 10 months after surgery. On CT examination 18 months after surgery, there was no evidence of the synthetic graft and instead, complete corticalization of the affected area was noted. Complete functional recovery was observed until the last clinical follow-up at 36 months post-operatively.

Conclusion: Screw fixation and use of a 3D-printed ceramic scaffold augmented with rhBMP-2 resulted in excellent bone regeneration of the non-union and full recovery of a miniature-breed dog.
**Introduction**

Fractures of the radius and ulna in toy- and miniature-breed dogs occur frequently as a result of minor trauma, having a higher risk of complications than have similar fractures in large-breed dogs. These complications include delayed union, non-union, osteopenia and re-fracture after implant removal. In the most severe cases, the above-mentioned complications can end up with critical-sized bone defects. Autologous cancellous bone-grafting is still considered the gold-standard solution for enhancing the healing of bone defects because it simultaneously provides osteoconductive, osteoinductive and osteogenic properties. This technique carries several drawbacks, noticeably the limited availability of cancellous bone.

Synthetic bone biomaterials based on calcium phosphate (CaP) have proved to be an adequate alternative to autologous bone grafts due to their structural and chemical similarity to the bone mineral phase, providing excellent osteoconductive properties. Recent advances in the bone-tissue engineering field have enabled the fabrication of CaP scaffolds representing an accurate replica of a specific bone defect using a technology called 3D-printing. Incorporation of growth factors or osteogenic cells to the bioceramic scaffolds improves bone regeneration by providing additional osteoinductive or osteogenic properties, respectively, to the scaffold. Among all of the growth factors, recombinant human bone morphogenic protein-2 (rhBMP-2) has been reported as being one of the most extensively used in both human and veterinary orthopedic surgery due to its powerful osteoinductive properties. The purpose of this report is to describe the successful treatment of a severe, radial atrophic non-union in a miniature dog by using an innovative 3D-printed beta-tricalcium phosphate (β-TCP) synthetic bone graft combined with rhBMP-2.

**Methods**
A one-year-old male Yorkshire terrier, weighing 3.7 kg, was referred to our center with a history of non-weight-bearing lameness of the left front limb. The dog had suffered a distal left radial fracture treated surgically two months earlier with a 2.0 locking compression plate (LCP) with 8 combi-holes (Depuy Synthes Vet.; Pennsylvania, USA), which can accommodate standard or locking head screws in the same hole (Figure 1A&B). The referral veterinary surgeon confirmed that it was a closed fracture and that no signs of infection were observed in the animal along the post-operative period. No trauma occurred following surgery according to the owners. Both the referral veterinary surgeon and the owner confirmed that the dog had used the operated leg during the two or three weeks after the surgery and had started to show some lameness around one month after surgery. On presentation, the dog only revealed the non-weight-bearing lameness of the left forelimb. Under sedation, mediolateral and craniocaudal radiographs of the affected limb were taken. The radiographs showed a large radius bone defect of 2.3 cm in length, representing about 25% of the total radius length. Radiographic findings were consistent with a severe atrophic non-union with osteopenia and bone resorption of the mid/distal radius (Figure 1C&D).

A computed tomography (CT) of both forelimbs was performed and the CT data of the healthy right radius were used as a reference to produce the 3D-printed β-TCP scaffold replica of the left radius bone defect. DICOM data from the CT can be converted to a Computer-Aided Design (CAD) file which can be processed by the computer with CAD software (Solidworks 2014, Dassault Systèmes SolidWorks Corp.; Waltham, MA) and sent to the 3D-printer (Pastecaster, BCN3D Technologies; Barcelona, Spain). The scaffold was printed 4 mm longer than the original radial bone defect to guarantee a proper press-fitting fixation after some degree of surgical curettage of the defect bone-ends. The process of CaP scaffolds fabrication by 3D robocasting is well described in
Briefly, the customized scaffold was constructed layer-by-layer using a direct ink-writing printer by microextrusion of a ceramic (α-TCP) suspension in a hydrogel (poloxamer 40730 wt% solution in de-ionized water) at a constant volumetric flow rate, creating uniform strands of pre-selected diameter (250 µm) in an orthogonal pattern. After printing, the scaffold was left to dry in air overnight, and afterward it was heat-treated to 400ºC for 5h and, subsequently, to 1100ºC for 3h. As a result, a macroporous β-TCP scaffold was obtained, with a compressive strength of 13 MPa, 54% of macroporosity and open and interconnected prismatic-shaped macropores with pore dimensions of around 200 µm. Sterilization was conducted in an autoclave at 121ºC, 2 atm for 20 minutes.

For the surgical procedure, the dog was premedicated with medetomidine (2 µg/kg intramuscularly [IM]) and methadone (0.2 mg/kg IM). General anesthesia was induced with propofol (2 mg/kg intravenously [IV]) and diazepam (0.5 mg/kg IV) and maintained with a gas mixture of isoflurane (1.5%) and oxygen in a non-rebreathing system. Cefazolin sodium (22 mg/kg IV) was administered after induction. The diaphyseal area of the left radius was exposed using a craniomedial surgical approach. The two central loose-locking screws of the plate, located in the bone defect area, were removed. The second proximal 2.0 locking screw, which was also unstable, was changed for a 1.5 standard cortex screw reoriented proximally, and an additional 2.0-mm standard cortex screw was placed in the most proximal hole of the plate. Curettage of the bone-ends was performed. The β-TCP construct was easily hand-modeled with a scalpel to assure a suitable press-fit of the scaffold in the bone gap (Figure 2). Two 2/0 monofilament, synthetic absorbable sutures were placed at two different levels surrounding the synthetic graft and the bone plate. Two absorbable collagen sponges impregnated with 0.66 mg (0.2 mg/mL) of rhBMP-2 (TruScient, Zoetis; Parsippany,
NJ) were prepared intraoperatively. Before implanting the collagen sponges longitudinally at both sides of the β-TCP construct, the sponges were first softly squeezed to soak the β-TCP scaffold. Closure of the surgical incision was performed routinely in layers. A supporting bandage (Robert-Jones) was placed on the limb for the following two weeks. The dog was discharged the day following the surgery with meloxicam (0.1 mg/kg orally, once daily) and cephalaxin (22 mg/kg orally, twice daily) for 10 days and tramadol (2 mg/kg orally, once daily) for five days.

**Results**

Post-operative radiographs showed an appropriate positioning of the scaffold and an adequate placement of the screws (Figure 3). Although the image can give the impression of insufficient contact between the bone and the implant at the proximal end, this is actually a false appearance caused by the monocortical end of the proximal bone fragment. Two weeks after surgery, the dog had already recovered close-to-normal weight-bearing of the affected limb, with a mild lameness that completely disappeared two weeks later. A monthly radiographic post-operative assessment revealed a progressive remodeling and biodegradation of the scaffold with simultaneous progressive bone regeneration through the scaffold (Figure 3). Complete healing of the radius defect was observed radiographically at four months after surgery. Ten months after surgery, the bone plate was removed to allow for complete functional load along the regenerated radius and to avoid the potential risk of stress shielding (Figure 4A). Subsequent radiographic follow-ups showed progressive filling of the screw holes and an advanced bone remodeling, giving the affected radius an almost normal aspect (Figure 4B, C). Eighteen months after surgery, a CT examination was performed and showed no traces of the robocasted graft, and a complete corticalization
of the regenerated bone area (Figure 4D, E). Complete functional recovery was observed until the last clinical follow-up performed 36 months after surgery.

Discussion

The high complication rate in the treatment of radius fractures in toy- and small-breed dogs can be associated with both biomechanical and vascular aspects. On the one hand, biomechanical factors, such as increased stiffness and reduced interfragmentary strain following bone-plating, seem to contribute to the high incidence of osteopenia and delayed union. On the other hand, low microvascular density in the distal bone diaphysis in toy breeds and poor soft tissue coverage of the distal antebrachium, which limits the capacity of these tissues to provide extraosseous circulation to the fractured bone, may also hamper bone healing. In our dog, we consider that a combination of an insufficient blood supply response and mainly a too-stiff plate creating a stress-shielding effect were the origin of the bone resorption. As bone resorption progressed and involved the bone area where the second and third screws were placed, the proximal fragment became clearly unstable, and that instability was the reason for the dog’s lameness at presentation.

Bone healing is a very complex phenomenon and most of the times a successful outcome depends on the simultaneous presence of several factors (bone fragments stability and reduction, vascular supply, cellular response, etc.). The additional placement of two screws in the proximal bone fragment provided enough interfragmentary stabilization to allow the animal to walk again without lameness just after the surgery and to provide an adequate mechanical environment for bone healing. Nevertheless, considering the size of the bone defect and the atrophic nature of the non-union, the stability provided by the screw addition was clearly insufficient to assure bone healing, therefore biological stimuli had to be provided in the bone defect area.
When a very limited amount of fresh cancellous bone is available, such as in miniature dogs, synthetic osteoconductive and/or osteoinductive products can provide the above-mentioned biological stimulation. The effective clinical use of β-TCP as a synthetic osteoconductive bone substitute has been largely demonstrated in veterinary orthopedics, usually as particulates or granules. However, these presentations have important drawbacks such as graft migration and poor mechanical properties, and thus, limited osteoconductive capacity. In this regard, 3D-printing technology has emerged to develop complex geometrical bone substitutes with high precision and accurate control of the internal architecture and the outer shape of scaffolds to improve their mechanical properties and osteoconductive or even osteoinductive potential. Moreover, and based on CT-imaging data files of patients, current 3D-printing technology applied to orthopedics enables a rapid fabrication of personalized bone substitutes customized to the shape of the patient’s bone defect and its clinical condition, notably reducing the bone defect healing time.

Regarding the use of rhBMP-2, and despite its high efficacy as an osteoinductive factor, the sustained and local delivery of rhBMP-2 using a suitable carrier is essentially required to accelerate bone healing. Recently, a variety of BMP-2 delivery systems based on 3D-printed scaffolds have been investigated in experimental studies, obtaining promising results in terms of bone healing. However, to our knowledge, the case described herein is the first veterinary clinical report using 3D-printing technology with rhBMP-2 for the treatment of a large bone defect. The clinical case presented in this paper is a clear example of the synergy between the carrier and the bioactive molecule. The presence of the β-TCP osteoconductive scaffold avoided the uncontrolled formation of a hypertrophic callus observed in some experimental and clinical cases treated with rhBMP-2 and properly guided bone regeneration, leading to the full-
thickness cortical-bone bridging. In addition to permitting the proliferation of osteogenic
cells and vascular in-growth, calcium-phosphate scaffolds are known to have a high
affinity for osteoinductive proteins allowing, in our case, to retain the BMP molecules
in the defect site and to prevent their fast diffusion to adjacent tissues. Finally, the
ceramic scaffold was demonstrated to be biodegradable with a progressive resorption
kinetic closely connected with the advancing osteogenic front protecting BMPs from
degradation for a sufficient period of time.

In conclusion, the entire follow-up of the clinical case presented herein demonstrates
that the combined screw addition and use of a 3D-printed ceramic scaffold and rhBMP-
2 provided excellent bone regeneration, allowing for the complete healing of a critical-
sized bone-defect non-union affecting the dog in our case. The therapeutic approach
used in this dog could be an option to be considered for the treating of large-bone
defects in veterinary orthopedics and, especially, for those affecting the distal radius of
miniature dogs.

**Disclosure Statement**

The authors have no conflicts of interest to declare for this report.

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The English of this manuscript has been proofread by Mr. Chuck Simmons, a native
English-speaking University Instructor of English.
References


Figure Legends

Figure 1. Immediate post-operative mediolateral (A) and craniocaudal (B) radiographs: previous fracture repair with a locking compression plate, 2 months prior to presentation. Mediolateral (C) and craniocaudal (D) radiographs at presentation. Note the severe bone defect and appearance consistent with atrophic non-union.

Figure 2. Intra-operative image: the synthetic β-TCP scaffold is press-fitted in the radius gap under the pre-existing plate.

Figure 3. Postoperative mediolateral radiographs of the affected radius obtained immediately after surgery and five months later. Note the progressive healing of the bone defect.

Figure 4. (A) Mediolateral radiograph after plate removal at 10 months post-surgery. Mediolateral (B) and craniocaudal (C) radiographs 18 months after surgery, consistent with complete bone healing of the affected area. (D) CT 3-D reconstruction of both forelimbs 18 months after surgery. (E) CT section of the distal third of the left radius (up). No evidence of β-TCP scaffold remnants was found. Instead both radii appeared similar on CT sections obtained at the same level (bottom).
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