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Comparison of the Osteogenic Potential of OsteoSelect Demineralized Bone Matrix Putty to NovaBone Calcium-Phosphosilicate Synthetic Putty in a Cranial Defect Model

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Abstract: The purpose of this study was to compare the osteogenic potential of a synthetic and a demineralized bone matrix (DBM) putty using a cranial defect model in New Zealand white rabbits. Paired, bilateral critical-size defects (10 mm) were prepared in the frontal bones of 12 rabbits and filled with either OsteoSelect DBM Putty or NovaBone calcium-phosphosilicate putty. At days 43 and 91, 6 rabbits were killed and examined via semiquantitative histology and quantitative histomorphometry. Defects filled with the DBM putty were histologically associated with less inflammation and fibrous tissue in the defect and more new bone than the synthetic counterpart at both time points. Histomorphometric analysis revealed that the defects filled with DBM putty were associated with significantly more bone formation at day 43 (70.7% vs 40.7%, $P = 0.043$) and at day 91 (70.4% vs 39.9%, $P = 0.0044$). The amount of residual implant was similar for both test groups at each time point.

Key Words: Bone regeneration, calvarial defect, demineralized bone matrix, bone graft substitute, DBM putty

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Surgical repair of defects in the skeletal system has proven to be one of the most fruitful endeavors in the field of regenerative medicine. Bony defects originating from trauma, infection, oncologic resections, developmental anomalies, and pathological deterioration are treated with bone grafts in more than 1.5 million Americans every year.[1] The clinical success of these bone grafts is made possible, in part, by the intrinsic capacity of bone to regenerate across a void if an appropriate scaffold is present to support bone ingrowth.[2] These scaffolds include autografts (host-derived bone), allografts (cadaver-derived bone), and various materials of purely synthetic origin or combined with a xenogeneic collagen.[3]

Autogenic bone is currently considered the criterion standard in bone grafting procedures because it contains the 3 elements critical to bone remodeling and repair: osteoconductivity, osteoinductivity, and osteogenetic properties. Osteoconductivity is inherent to all types of bone grafts and is best described as a scaffold for cellular attachment and proliferation. Osteoinduction describes the signaling required for cellular differentiation of osteoprogenitor cells into osteoblasts and osteoclasts. Bone morphogenetic proteins (BMPs), a member of the transforming growth factor β family, are signaling proteins present in bone known to be responsible for osteoinductive cellular differentiation.[4,5] Osteogenesis refers to cellular formation and development of bone through the action of differentiated bone-forming cells. Despite a high degree of efficacy associated with the use of autograft, this approach is subject to important limitations due to supply constraints, donor-site morbidity, increased operating and patient recovery time, incidence of complications, cost associated with harvesting and preparing the material, and, in some applications, anatomical shape of the harvested bone.[6–8] These limitations have led to the development and increased clinical use of allograft and synthetically derived bone graft substitutes.

Demineralized allograft bone has many of the desirable attributes of autograft bone. Once demineralized, the particulate demineralized bone matrix (DBM) is frequently combined with other components (referred to as "carriers") intended to make the DBM easier to handle in a clinical setting.[9] Despite the presence of an additional carrier material, these products are often referred to generically as DBM. Demineralized bone matrix products have been shown through clinical and animal testing to be effective in the regeneration of a variety of osseous defects in the extremities, pelvis, spine, cranium, and midface.[4,7,8,10–12] The demineralization process exposes the collagen and native growth factors, including the naturally occurring spectrum of BMPs, present within human bone, which have been shown to provide the osteoinductive properties of these materials.[13–18] Demineralized bone matrix can be processed by a variety of methods to produce a variety of shapes, sizes, and forms including blocks, strips, powders, gels, and pastes to accommodate the needs of different bone grafting procedures.

Synthetic materials have also been developed for use as bone graft substitutes. Advantages of synthetic materials include tunable resorption rates, increased mechanical strength compared with DBM

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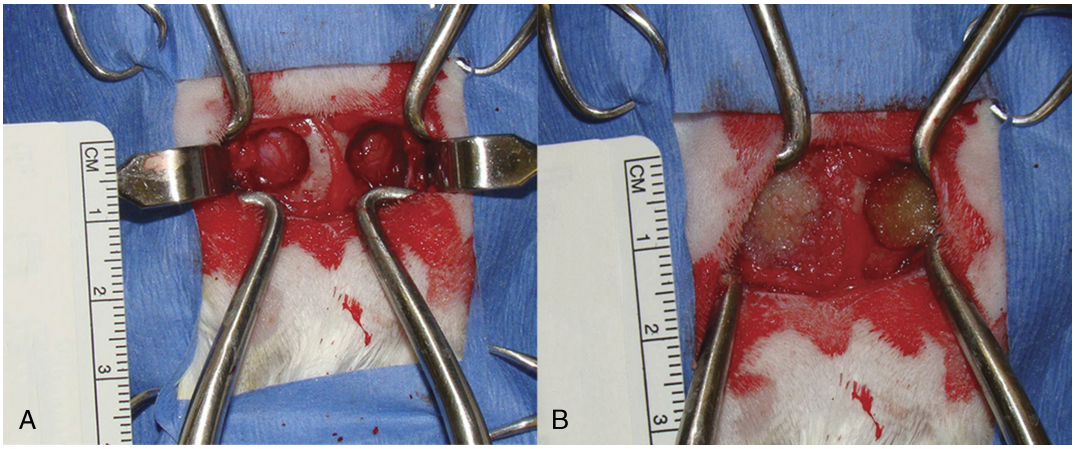

FIGURE 1. Picture of surgical implantation. A, Empty cranial defects. B, Filled defects (left side = OsteoSelect, right side = NovaBone).

products, controlled porosity, and ideal processing and molding parameters.[19,20] Although these materials have been shown to possess regenerative effects, the inherent lack of native growth factors leads to an absence of osteoinductive properties.[21]

While the cited studies clearly demonstrate the regenerative potential of DBM and synthetic bone graft substitutes, studies aimed at directly comparing the activity of the 2 different classes are limited. This present study sought to compare the regenerative potential of a DBM putty (OsteoSelect DBM Putty; Bacterin International, Inc [Belgrade, MT]) to a synthetic calcium-phosphosilicate putty (NovaBone Putty; NovaBone Products, LLC [Alachua, FL]) in a critical-size calvarial bone defect in skeletally mature New Zealand white rabbits.

MATERIALS AND METHODS

All aspects of the study detailed herein were performed by the Biological Test Center (Irvine, CA) under sponsorship from Bacterin International, Inc (Belgrade, MT). The animal research protocol was conducted in accordance with all Biological Test Center animal welfare policies and was approved by the Biological Test Center Institutional Animal Care and Use Committee. Twelve skeletally mature female New Zealand white rabbits at least 12 weeks old and weighing 4.0 to 4.8 kg (Western Oregon Rabbitry [Philomath, OR]) were randomly divided into 2 groups (groups A and B). Animals were anesthetized with an intravenous injection of ketamine (7.7 mg/kg) and xylazine (2.3 mg/kg). Anesthesia was maintained during surgery by the administration of isoflurane via inhalation as needed. The cranium of each animal was shaved, disinfected with Betadine and alcohol scrubs, and surgically draped using aseptic technique. A midline skin incision was made to expose the muscle fascia and periosteum of the sagittal crest. The temporalis muscle was retracted, and 2 bilateral, full-thickness craniotomies (approximately 10 mm in diameter) were created in the frontal bone using a hand trephine. Each animal served as its own control with one of the defects being filled with NovaBone Dental Putty (NovaBone Products, LLC) and the contralateral defect being filled with OsteoSelect DBM Putty (Bacterin International, Inc) (Fig. 1). Caution was used to avoid excessive compression during insertion of the bone graft substitutes into the defects. The pericranium and skin were closed in layers using nonabsorbable sutures, and the rabbits were allowed to recover. Following placement of Elizabethan collars, the animals were returned to their individual cages and monitored twice daily for mortality and morbidity. Buprenorphine (0.03–0.05 mg/kg) was administered subcutaneously as needed for pain management.

NovaBone Dental Putty was used without modification according to the manufacturer's instructions for use. The OsteoSelect DBM Putty was prepared from demineralized rabbit bone and mixed with a bioabsorbable carrier (carboxymethylcellulose) and phosphate-buffered saline to form a putty-like consistency. With the exception of the use of rabbit DBM, the formulation and process used to produce OsteoSelect DBM Putty were the same as those used to produce commercially available OsteoSelect DBM Putty.

On day 43 (group A) or day 91 (group B), the animals were humanely killed by an intravenous bolus injection of commercially available euthanasia solution. The crania were surgically removed and fixed in 10% neutral-buffered formalin. Three sections were made from each filled defect in a sagittal plane at the midpoint and approximately 2.5 mm lateral and medial to the midpoint of the defect. The sections were processed to slides and stained with hematoxylin and eosin. Histology was performed on each section to evaluate inflammation, the amount of residual implant material, the amount of fibrous tissue in the defect, the extent of bridging of the defect by new bone formation, and the quality of the new bone. Histology was graded on a semiquantitative scale of 0 to 3 as outlined

TABLE 1. Histology Scoring Matrix

Parameter	Score
Inflammation	3—Severe
	2—Moderate
	1—Mild
	0—None
Amount of residual implant material	3—Large
	2—Moderate
	1—Small
	0—None
Amount of fibrous tissue in the defect	3—Marked
	2—Moderate
	1—Mild
	0—None
Defect bridged by new bone formation	3—Complete
	2—Moderate with small gaps
	1—Little stumps
	0—None
Quality of new bone	3—Lamellar bone
	2—Mixed woven and lamellar bone
	1—Mainly woven bone
	0—No bone

in Table 1. Following histology, quantitative histomorphometry was performed under 20× magnification using a Motic Digital Slide Scanner and a Motic Automated Tele-Microscope Model BA600 MOT (Motic Corporation Ltd, Hong Kong) for each section to quantitatively determine the amount of new bone formation and residual implant within each filled defect. All histology was performed by an independent laboratory (Vet Path Services, Inc [Mason, OH]). Histopathology evaluation and histomorphometry measurements were conducted on blind sections by an independent board-certified veterinary pathologist.

The ratio of new bone formation and residual implant for the NovaBone-filled defect and the OsteoSelect-filled defect was calculated for each animal using the histomorphometric measurements. *Z* scores and *P* values were calculated from the mean ratio compared with the null hypothesis of equal bone formation and residual implant for the 2 different bone graft substitutes. For the histopathology results, 2-sided *t* tests were performed on the raw semiquantitative data. $P < 0.05$ was considered to be statistically significant.

RESULTS

Semiquantitative histopathology revealed differences between cranial defects filled with NovaBone Putty and those filled with OsteoSelect DBM Putty in day 43 and day 91 rabbits. The results are shown in Figure 2. At day 43, the amount of inflammation ($P = 0.0001$), residual implant material ($P = 0.034$), and fibrous tissue ($P = 0.0041$) were all significantly higher for NovaBone Putty than for OsteoSelect DBM Putty. The extent of bridging of the defect by new bone was higher for OsteoSelect ($P = 0.0013$). The quality of bone was the same for both groups ($P = 1.00$). At day 91, inflammation ($P < 0.0001$) and the amount of fibrous tissue in the defect ($P = 0.0003$) were all significantly higher for NovaBone Putty than for OsteoSelect DBM Putty. The extent of bridging of the defect by new bone was higher for OsteoSelect ($P = 0.0038$). The amount of residual implant material ($P = 1.00$) and the quality of bone ($P = 1.00$) were the same for each group.

Following histological examination, the defect sites were examined histomorphometrically for quantitative evaluation of residual implant and new bone formation. The results are shown in Figure 3. At day 43, OsteoSelect DBM Putty induced formation of considerably more new bone than did NovaBone Putty (70.7% vs 40.7%, $P = 0.043$). The amount of residual implant was similar for both groups (17.7% vs 29.1%, $P = 0.21$). At day 91, OsteoSelect DBM Putty induced considerably more new bone formation than did NovaBone Putty (70.4% vs 39.9%, $P = 0.0044$). The amount of residual implant was similar for both groups (19.0% vs 19.5%, $P = 0.30$). The remainder of the defect was composed primarily of connective tissue with or without inflammatory cell components. Representative histology slides are shown in Figures 4 and 5. At 43 and 91 days, defects filled with OsteoSelect DBM Putty were histologically characterized by small, isolated fragments of residual implant material surrounded by a combination of new cortical and trabecular bone growth that spanned the full width of the defect area. Defects filled with NovaBone Putty demonstrated incomplete bone ingrowth across the defect area, accompanied by large, solid regions of residual implant material.

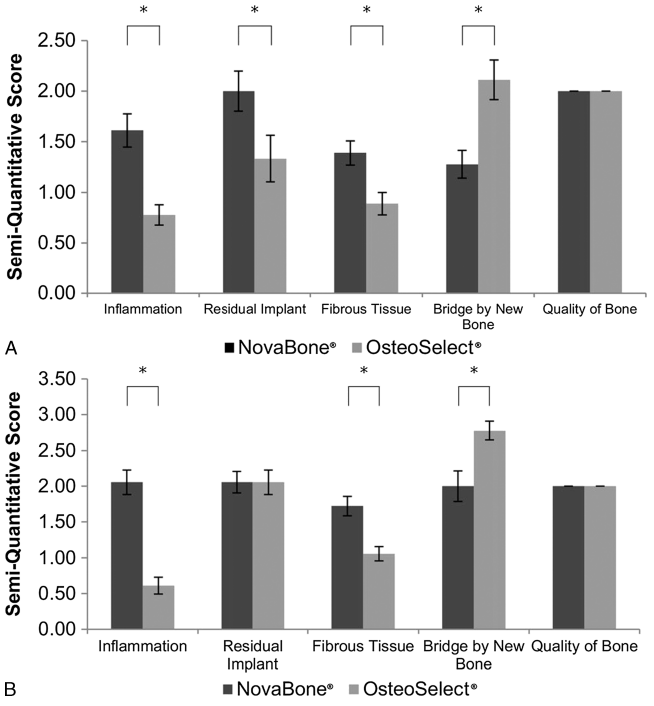

FIGURE 2. Semiquantitative histology results. The graphs represent averages and SEs for each metric examined. A, Day 43 results. B, Day 91 results. Statistically significant results shown with an asterisk.

DISCUSSION

The objective of the study was to evaluate the osteogenic potential of a DBM putty in comparison to a synthetic putty when implanted in a critical-size bone defect in the crania of New Zealand white rabbits. On days 43 and 91, OsteoSelect DBM Putty was associated with less inflammation, less fibrous tissue, greater bone formation, greater defect bridging, similar levels of residual implant material, and similar new bone quality when compared with NovaBone Putty, as determined by semiquantitative histopathologic and quantitative histomorphometric evaluation.

To evaluate the clinical implications of these findings, it is instructive to compare the present results with those previously reported. In 2002, Clokie and colleagues[22] compared the regenerative effect of a DBM putty to calcium sulfate pellets and 2 different calcium phosphate cements in critical-size calvarial defects in New Zealand white rabbits. The rabbits were examined histomorphometrically at 6 and 12 weeks postoperatively for new bone formation. The DBM putty showed 10.9 times more new bone formation than the best performing synthetic at 6 weeks and 3.0 times more at 12 weeks. These results show that not only did the DBM putty result in more new bone formation but also more rapid formation of new bone than any of the 3 synthetics. In addition, in 2011, Khoshzaban and colleagues[23] compared the regenerative effect of a DBM to a synthetic bone graft substitute, β-tricalcium phosphate, in critical-size calvarial defects in Wistar rats. In their study, the DBM graft showed 3.7 times more new bone formation at 4 weeks. This result was confirmed at 10 weeks when the DBM graft showed 2.0 times more new bone formation compared with the synthetic bone graft substitute. Furthermore, several additional studies have been reported in which DBM products outperform synthetic bone substitutes in terms of new bone growth.[24,25]

The enhanced bone formation of the DBM putties in these studies is likely a reflection of their osteoinductive potential.[26] Although the exact spatial and temporal sequences of bone remodeling and repair are still being uncovered, it is certain that the process is highly coordinated, involving numerous signaling events and multiple cell types.[27] While both synthetic and DBM putties provide a scaffold for bone formation and blood vessel ingrowth, BMPs and other growth factors within demineralized bone have been reported to induce the differentiation of osteoblast progenitor cells.[28]

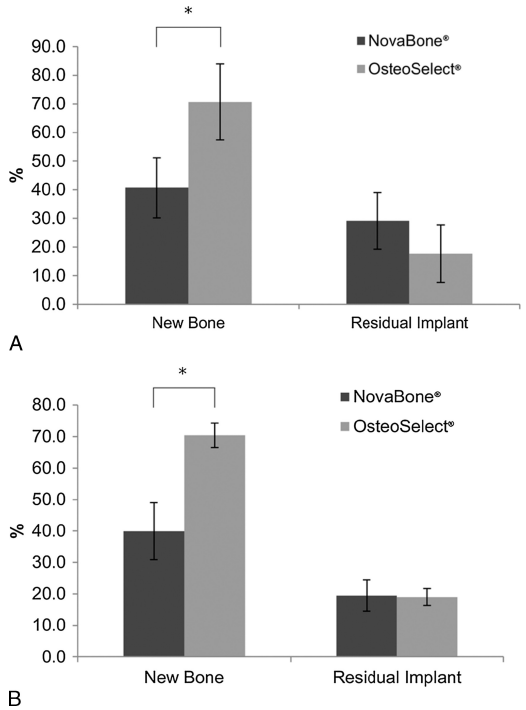

FIGURE 3. Quantitative histomorphometry results. The graphs represent averages and SEs for each metric examined. A, Day 43 results. B, Day 91 results. Statistically significant results shown with an asterisk.

This cell differentiation likely enhances bone remodeling and graft integration, thus providing a pronounced advantage when using DBM, which unlike synthetics maintains bone's natural regenerative capacity.

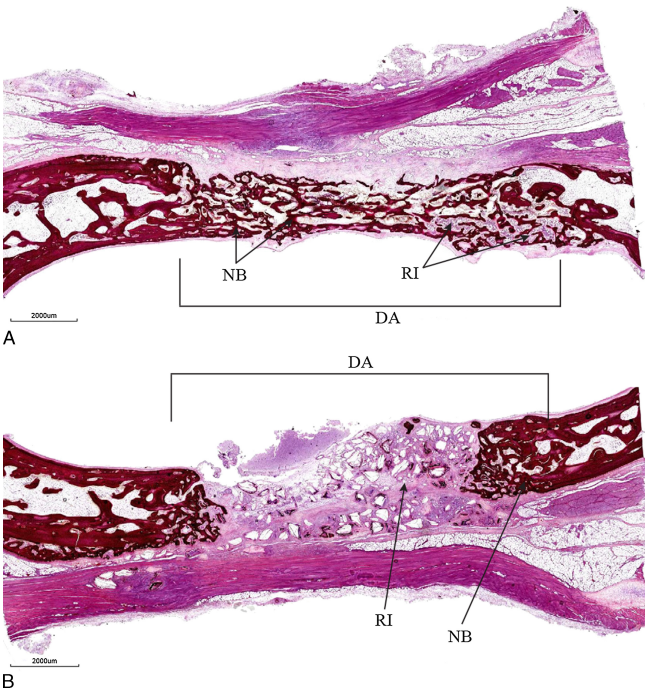

FIGURE 4. Representative histology slides from the 2 bony defects in the same animal at day 43 for (A) OsteoSelect and (B) NovaBone showing the defect area (DA), residual implant (RI), and area of new bone formation (NB). Slides are stained with hematoxylin-eosin and shown under 10× magnification.

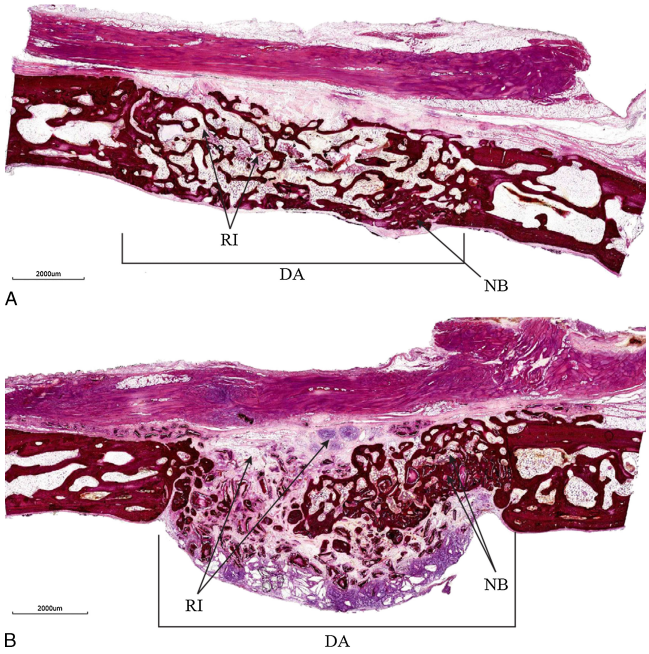

FIGURE 5. Representative histology slides from the 2 bony defects in the same animal at day 91 for (A) OsteoSelect and (B) NovaBone showing the defect area (DA), residual implant (RI), and area of new bone formation (NB). Slides are stained with hematoxylin-eosin and shown under 10× magnification.

The results generated in this study, coupled with previous animal models presented in the literature, suggest that DBM putties are superior to synthetic bone graft substitutes in terms of bone regeneration of cranial defects. The long-term clinical implications of this enhanced regenerative capacity are the subject of ongoing study, and additional studies, including studies in humans, are needed to determine the full clinical significance of these findings.

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