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Evaluation of an osteostimulative putty in the sheep spine

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Abstract The objective of this study is to evaluate local response to a bioactive glass based composite putty (NovaBone Putty) in a vertebral body defect model in sheep, as compared to NovaBone, a bioactive glass particulate. Two time periods were used for the study, 6 and 12 weeks. Empty defects were also used as a control. In comparing the three test groups, the relative amount of new bone for both grafted defects was substantially greater than for the empty controls ($P < 0.05$). At 6 weeks, the bone formation was 42% for NovaBone Putty, 27% for NovaBone and 1.2% for the ungrafted empty defect. At 12 weeks, the bone formation was 51.4% for NovaBone Putty, 47.3% for NovaBone and 5.1% for the empty defect. NovaBone Putty, the test material, had greater bone content than the NovaBone, both of which were significantly greater than the empty control.

1 Introduction

Bone fractures and other orthopedic defects often require augmentation with some type of graft material to achieve full healing. It is estimated that in the USA alone, over 500,000 bone graft procedures are conducted annually, with roughly half of these procedures being conducted in the spine.

Autogenous bone is historically regarded as the graft of choice, it being both osteoconductive and containing autologous inductive factors and proteins. However, its use is often complicated by its limited supply and donor site morbidity. Allograft bone may be used as an alternative, but while limiting the donor site morbidity of autografts, it also has drawbacks. These include supply limitations, partial loss of osteoinductivity due to graft tissue harvesting and treatment, and concerns involving disease transfer.

Due to the drawbacks associated with the tissue grafts, various synthetic bone graft substitutes have been developed. Among these synthetics is the family of calcium phosphate ceramics [1] and glasses [2], calcium phosphates being selected due their chemical similarity to the mineral phase of bone. By being chemically similar to bone mineral, some of these materials actually provide a framework upon which direct bone apposition and bonding may occur.

One such synthetic material is the calcium phosphosilicate [2–4] based NovaBone calcium phophosilicate materials are in the class of bioactive glasses and have been reported to release ions in physiological environment [5, 6], activate osteoblast gene expression [7, 8] and enhance the osteoblast proliferation [9–15]. This osteostimulation results in new bone formation throughout the grafted site as rates faster than those seen with other synthetics [16]. Previous animal studies demonstrated that these bioactive materials are resorbable and enhance new bone growth significantly [17, 18]. The material, which is in particulate form, can be used alone or as an extender for autograft bone, reducing the need for extensive autogenous bone harvesting. However, in clinical situations, the handling of the bone graft materials is a critical issue, which may limit the application of the materials. Therefore, it is

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of importance to develop different form of the implant materials to meet the requirement for different clinical situations. In addition, some in vivo studies showed that, bioactive glass particulates did not stimulate bone formation in some cases due to the migration of the glass granules, which sometimes also associated with certain degree of inflammation [19–21].

The goal of this study was to evaluate whether the inclusion of a resorbable carrier as a handling aid for the particulate bone graft would impact the amount of bone growth into an osseous defect. Critical-size defects in the sheep spine were used, with the amount of new bone formation into these defects being compared 6 and 12 weeks after implantation. In order to obtain more accurate comparison, a modification of the animal model was made by covering the defects with bone discs to prevent form possible migration of the bioglass particulates.

2 Materials and methods

Uniform surgical defects were created in three adjacent lumbar vertebrae in sheep. The defects each received one of three treatments, involving the test material (NovaBone with carrier), NovaBone bioglass particulates, and an empty defect. All three treatments were applied in each animal. Twelve animals were sacrificed, six at 6 weeks and the other six at 12 weeks. At sacrifice, the vertebral bodies were harvested and evaluated histologically for new bone formation and for residual graft material content.

The test material (PUT) was a synthetic device having a putty-like consistency. The PUT material consisted of the calcium phosphosilicate particulate in a gel-like binder containing glycerin and polyethylene glycol. NovaBone (NB) consisted of the same particulate, but without the carrier. The empty group (EMP) was the empty defect.

- PUT—Bioglass® Putty (NovaBone Products, Alachua, FL)
- NB—Bioglass® (NovaBone Products, Alachua, FL)
- EMP—Empty defect, no graft material

Twelve sheep (male, Mianyang sheep, approximately 2.5 years old, ranging in weight from 110 to 150 lb) were implanted in this study. All animals were identified by ear tags or similar permanent markings. Animals were quarantined for a minimum of 1 week prior to surgery. All animals were housed in communal 4 m × 4 m pens, seven animals per pen. Two weeks after surgery, the animals were transferred to a larger outdoor pasture. All animals were fed a standard grass-alfalfa hay diet. Water was provided ad libitum. Animals were fasted at least 12 h prior to surgery.

The animals were sedated and anesthetized using intramuscular general anesthesia. Records of the type and amount of anesthesia administered were maintained. The animal was placed in right lateral recumbency and the left lateral lumbar region was clipped and shaved, prepared with betadine and alcohol scrubs, and draped using aseptic surgical techniques. Using a left lateral retroperitoneal approach, a skin incision was made to adjacent the lumbar spine. The psoas muscles were retracted to expose the third, fourth, and fifth lumbar vertebral bodies.

Beginning with L3, a small round burr was used to trace a 10 mm diameter defect in the lateral cortical wall. The traced line then was extended through the cortex with the burr, resulting in the removal of a disk of cortical bone approximately 10 mm in diameter. This disk was saved and used later to cover the defect, after placement of the graft material. The defect was completed using drill bits of sequentially increasing diameters to a depth of 15 mm, resulting in final defect 10 mm in diameter and 15 mm deep. A slow-speed hand drill with copious irrigation was used to minimize the damage to the surrounding bone.

Once the defect was created, it was irrigated with normal saline and filled with the appropriate graft material. The graft materials were prepared for placement as listed in their directions for use. The PUT test material is supplied ready to use on opening the package and required no mixing prior to placement. The NB was mixed with approximately 0.8 ml of sterile saline to the 2.0 cc of material in the package.

Caution was used to avoid excessive graft compression during insertion of the materials into defects. In order to reduce the migration of the particulates, the 10 mm cortical bone disk removed during initial site preparation was replaced over the surgical defect to cover the site and retain the graft materials after filling of the lateral defects, using bone wax to maintain positioning of the cortical disk. The cover discs were collected in a way so that they were slightly larger than the defect holes, so that the discs were fitted very well on the top of the defects and were not possible to fall into the defect sites. Following grafting of all three sites, routine multi-layer suturing was performed. Absorbable sutures were used for the external muscular fascia and subcutaneous tissue, and monofilament non-absorbable sutures were used for the skin.

After surgery, animals were monitored until they recovered from the anesthetic. When the swallowing reflex was present, the animal was propped up in metallic frame to prevent regurgitation and aspiration of stomach contents. The animals were monitored throughout the day of surgery, after which they were returned to their pens. Animals were observed daily for general health, including monitoring of general health, behavior, and appetite. Incision sites were

evaluated for healing for the first 2 weeks. All animals were weighed before surgery and just prior to sacrifice.

On the designated euthanasia date, animals were weighed and radiographs of the surgical sites were taken. The animals then were sacrificed via an intravenous bolus of supersaturated potassium chloride used under the supervision of a veterinary surgeon. Following euthanasia, the lumbar spines were retrieved via sharp dissection and local tissues were examined. All the surrounding musculature was removed from the lumbar spine. The three operated vertebral bodies (L3, L4 and L5) were isolated and placed in 10% formalin in individually-labeled containers.

After initial fixation, the transverse and spinous processes were removed, keeping the samples moist using normal saline at all times during the cutting process. The vertebral body was then infiltrated and embedded in polymethylmethacrylate (PMMA) and processed for hard tissue (undecalcified) plastic embedding. Using the cortical defect in the left lateral cortical wall as a guide, sections approximately 500–1,000 μm in thickness were cut with a diamond saw through the graft site, perpendicular to the long axis of the vertebrae in the transverse plane. The sections were ground and polished to a thickness of 50–60 μm and stained with Van Gieson's stain with Stevenel's blue counterstain.

Qualitative histopathologic analysis was performed for evaluation of local tissue reactivity to the implant materials and the presence of the materials. Further quantitative analysis was carried out using LEICA Q win3.3 image analysis software. Histomorphometric measurements were conducted to evaluate: (1) The amount of new bone formed within the graft site, and (2) The content of residual graft material.

The bone formation results were analyzed via an analysis of variance to evaluate between the different groups within each time period. A post hoc Duncan multiple range test was used to distinguish differences between multiple groups. The evaluation of the amount of residual graft material was conducted using a paired Student's *t*-test to directly compare the graft materials within each period. An unpaired *t*-test also was used to evaluate for differences between 6 and 12 weeks for each material. A level of significance of $P < 0.05$ was used to determine statistical significance for all analyses.

3 Results

Healing and animal condition were unremarkable, and their being no complications were observed. All animals maintained original weight, without instances of significant loss of weight or appetite, or of irregular behavior. There were no instances of post-operative erythema or edema, and there were no deaths among the test animals. In addition, no abnormal soft tissue structures were seen adjacent to the graft sites at sacrifice. Figures 1 and 2 are histology pictures from a representative animal for each time period, showing the three test groups for each animal.

In order to compare the bone formation difference caused by two different materials more accurately, and to reduce the effect caused by the migration of the particulates, bone discs were used to cover the defect sites after implanting the materials into the defects. Since the cover discs were collected in a way so that they were slightly larger than the defect holes, the discs were fitted very well on the top of the defects and did not fall into the defect sites. The histology pictures (Figs. 1, 2) showed that, with this experimental design, no particulate migration and inflammation occurred. It is obvious that the cover discs did not involve in the bone formation, as the empty control did not show good new bone formation.

At 6 weeks (Fig. 1), the empty group showed minimal bone formation, with most new bone formation occurring at the defect margins. The NB group demonstrated significant amounts of new bone throughout the grafted area. The new bone, appearing red in the photomicrographs, was apparent on the surfaces of the particles, connecting the particles to one another. In some samples, less bone was seen at the central regions of the defects, but there were no samples that demonstrated the lack of bone as seen with the empty group. The PUT test material also demonstrated significant bone formation throughout the defect sites, similar to that seen with the NB group. Bone again spanned the gaps to connect adjacent particles by 6 weeks. The overall extent of bone formation appeared similar or greater than that observed for the NB group.

At 12 weeks (Fig. 2), little change was observed in the EMP group. Little new bone was observed, with the empty space filled with fibrous tissue. In the NB groups, the lamellar bone was observed, which indicated the maturation of the newly formed bone, with the bone more completely filling the spaces between particles (Fig. 2d). For the PUT material, the amount of bone in the graft spaces appeared similar to that seen at 6 weeks, again with lamellar bone filling the spaces between particles (Fig. 2f). Normal appearing marrow spaces were noted in regions not filled with bone, but due to the thickness of the histologic specimens, actual evaluation of the tissue/cells within the spaces was not possible.

For both the NB and PUT materials, the graft material there appeared to be resorption of the materials with time. At 12 weeks, cracks were apparent in the residual particles, with bone formed between the particle fragments (Fig. 2f). In addition, discrete layers of bone could be seen as having

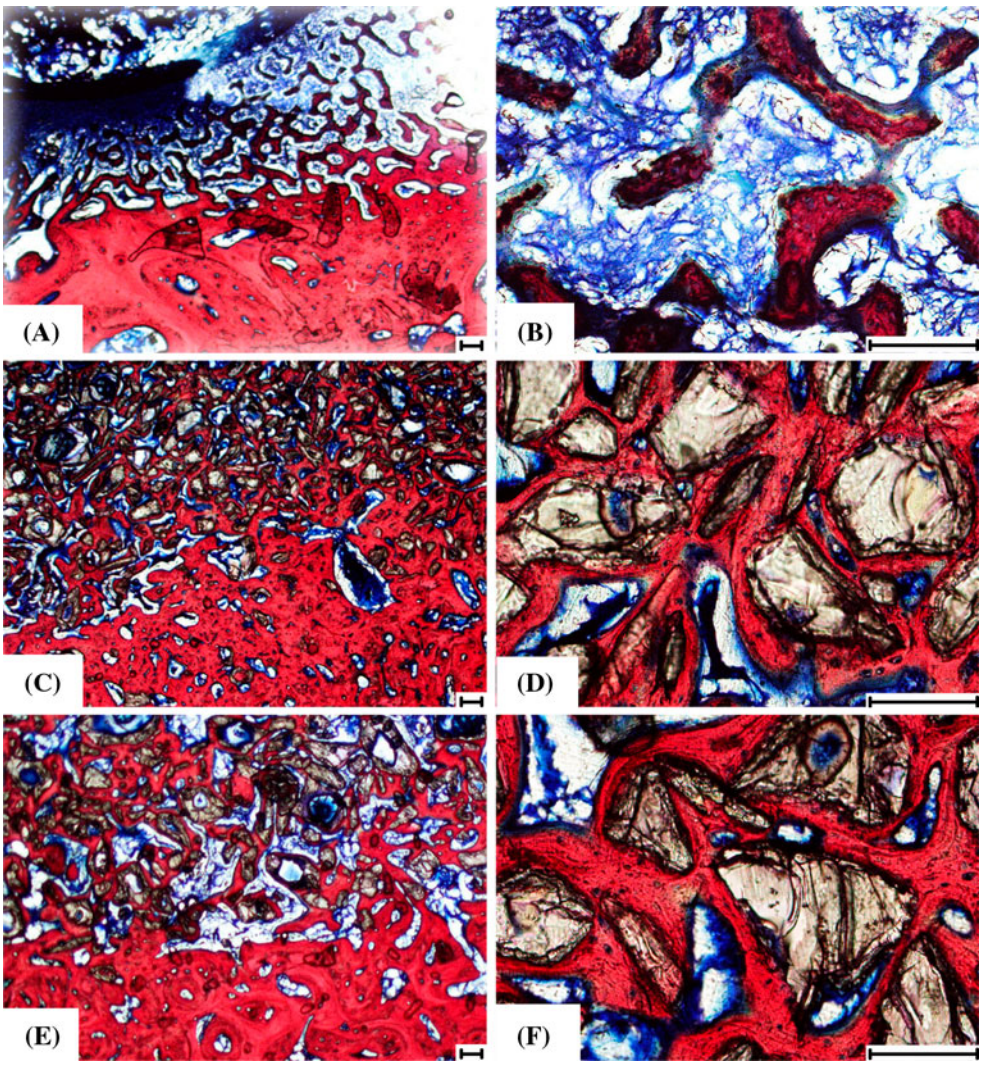

Fig. 1 Representative histology from sheep intravertebral implantation defects at 6 weeks; Animal #1. Van Gieson's stain with Stevenol blue counterstain **a** EMP negative control, **b** EMP negative control, **c** NB positive control, **d** NB positive control, **e** PUT test material, **f** PUT test material. *Bars* indicate 100 μm

been deposited on the particles, extending away from the particles as the bone grew in depth and maturity.

The amount of new bone and residual graft material were measured as a function of graft area. These data were recorded for each animal. The results are presented in Tables 1 and 2. In comparing the three test groups, the relative amount of new bone for both grafted defects was substantially greater than for the empty group ($P < 0.05$). At 6 weeks, the bone formation for the NB group was 20 times that of the empty defect. The increase in bone was even greater for the PUT test material, with a bone content of 42% versus just 1.2% for the empty group.

The NB group demonstrated the largest increase in new bone between 6 and 12 weeks, with an increase in average bone content of 74% over that measured at 6 weeks. While this was a significant change, the PUT test material still had significantly greater bone content than the NB group, both of which were greater than the EMP group.

In comparing the amount of residual graft material in the defects, the numbers were similar at each period. At 6 weeks, the differences were statistically significant, although the percentage of bone was very similar for both grafted groups, 33.6% for PUT versus 31.1% for NB. At 12 weeks, the residual graft content for both materials had decreased significantly, reaching almost identical value of 21–22% particulate.

4 Discussion

In this study, two similar graft materials were tested versus an empty group to determine their effect on bone formation

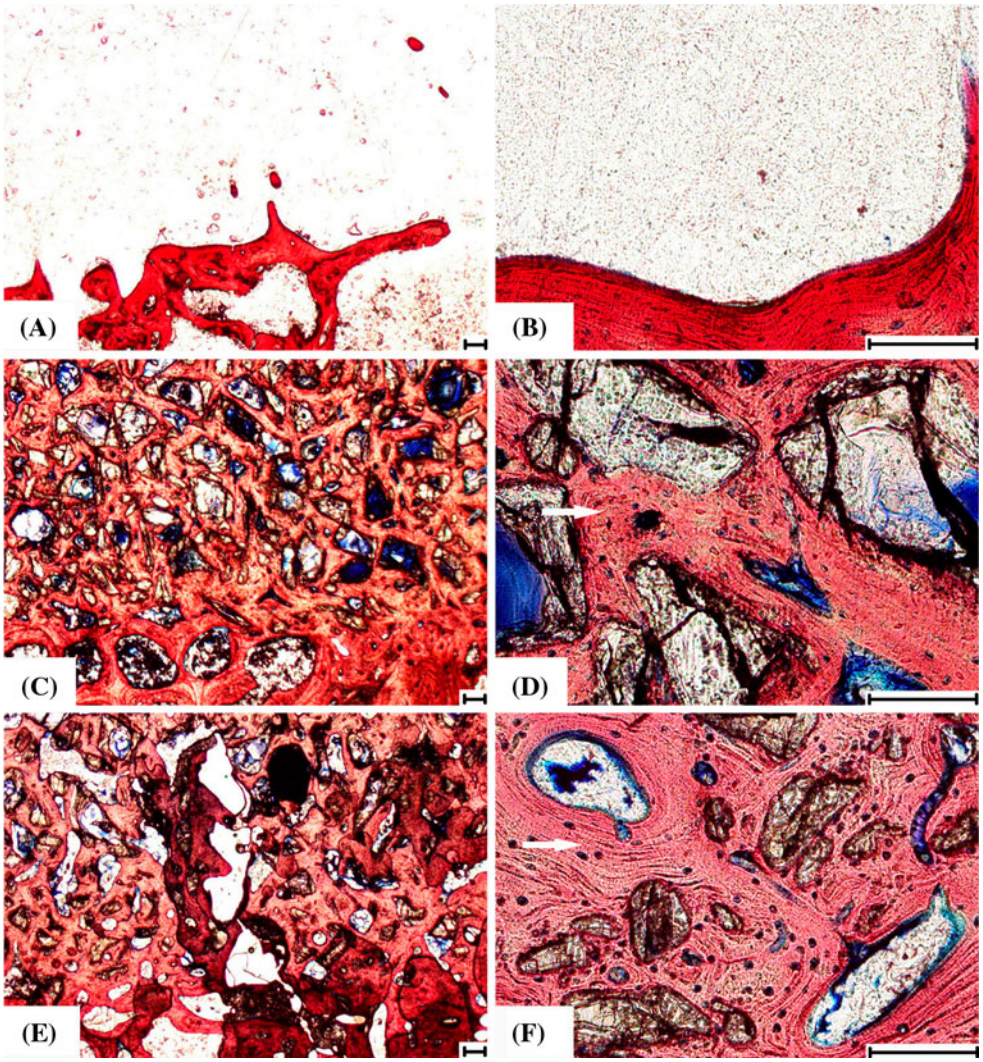

Fig. 2 Representative histology from sheep intravertebral implantation defects at 12 weeks; Animal #13. Van Gieson's stain with Stevenol blue counterstain. **a** EMP negative control, **b** EMP negative control, **c** NB positive control, **d** NB positive control, **e** PUT test material, **f** PUT test material. *Bars* indicate 100 μm

Table 1 Percent new bone content as a function of graft area (mean ± SD)

	PUT	NB	EMP
6 Weeks	42.0 ± 3.1[a,c]	27.0 ± 4.9[a,d]	1.2 ± 0.2[a,e]
12 Weeks	51.4 ± 1.8[b,c]	47.3 ± 2.8[b,d]	5.2 ± 1.0[b,e]

[a] PUT > NB > EMP at 6 weeks

[b] PUT, NB > EMP at 12 weeks

[c] PUT 12w > PUT 6w

[d] NB 12w > NB 6w

[e] EMP 12w > EMP 6w

Table 2 Percent residual graft material as a function of raft area (mean ± SD)

	PUT	NB
6 Weeks	33.6 ± 2.0[a,b]	31.1 ± 2.2[a,c]
12 Weeks	21.1 ± 4.0[b]	22.5 ± 4.1[c]

[a] PUT > NB at 6 weeks

[b] PUT 6w > PUT 12w

[c] NB 6w > NB 12 weeks

in a critical-size intravertebral defect in sheep. The two graft materials were similar in that their active ingredient was a bioactive glass particulate (NovaBone). The difference between the two materials consisted of the inclusion of an absorbable binder containing glycerin and PEG in the test material as a handling aid, the NB group not having such a binder. Both the PUT and NB group material filled the defect with new trabecular and lamellar bone, the amount of bone being significantly greater than that seen for the empty groups. By 12 weeks, the new bone occupied

approximately 50% of the defect area, with another 20% being occupied by residual graft material. In comparison, the empty controls only had an average fill of 5%, represented by new bone primarily along the defect margins.

This new bone formation for PUT and NB is consistent with previous studies [17, 18]. Bone formation was observed throughout the defect on the surfaces of the particles, bridging between the particles and anchoring them together as a three-dimensional lattice, supporting and stimulating further bone growth. That both materials reached a bone volume of approximately 50% is a testament to the efficacy of these materials. In addition, there was evidence that the materials were being gradually resorbed throughout the duration of the study. In another previous study [21], Kobayashi compared NovaBone Bioglass particulates with empty defect in a similar animal model and found that no significant difference of new bone formation between two groups. In that study, migration of the glass particulates were observed, which also associated with early stage inflammation after implantation. One critical difference of the experimental design between that Kobayashi's and the present study is that the bone defects were recovered using the bone discs removed during the creation of the defects. Our results showed that the migration of the particulates and associated early stage inflammation was prevented, and suggests that the glass granule migration is one critical factors which may affect the bone healing when using bioglass particulates.

The putty-like Bioglass had greater bone content than the particulate Bioglass at 6 weeks, while the amount of new bone was similar for both materials at the 12 week period. This would indicate that the initial presence of the binder may have actually helped to increase early bone deposition rates. The carrier used for this material is generally resorbed within 48–72 h. The possible explanation for the increased bone formation with the putty material at the 6 week time period could be that the addition of the carrier maintains a better spatial distribution of particles. During the initial healing period, the removal of the carrier through resorption allows for a better infiltration of bone precursor cells throughout the defect. It is clear from the histological observations at the 12 week time point that the carrier has no adverse effects on particle resorption or bone formation.

Our results suggested that the putty-like Bioglass had an advantage over particulates in clinical applications, especially for spinal procedures where bone grafting is necessary. In spinal fusions, for example, it is necessary that a graft material be able to maintain a structure, at least initially. The ability of the putty to be easily molded and hold together will prevent the graft dislocation and make the handling more convenient. Furthermore, due to its physical properties, the putty can be shaped so that bone defect can be filled easily with little residual implant migrating into unwanted areas. In addition, the increase of early bone deposition rate might also allow patients to start functional recovery training as early as possible.

5 Conclusions

Two synthetic bone graft materials, a bioactive glass putty and bioactive glass particulate, were compared versus an empty control in a critical-size intravertebral defect in sheep. The two graft materials were similar in that their active ingredient was a bioactive glass (NovaBone) particulate. The difference between the two materials was the inclusion of an absorbable carrier in the test material as a handling aid. The NovaBone Bioglass particulate, a clinically available particulate bone graft device, does not have such a carrier. The presence of the carrier gave the test device a putty-like consistency during use, which showed a clear handling advantage for surgical operation as compared with the particulates.

Both devices resulted in a filling of the defect with new trabecular and lamellar bone, the amount of bone being significantly greater than that seen for the empty controls. No migration of the particulates were observed due to the application of the bone discs on the top of the defects, suggesting that this technique was an effective way to prevent from particulate migration. At 6 weeks, the putty had greater bone content than the NB group. By 12 weeks, the new bone occupied approximately 50% of the defect area for both graft materials, with another 20% being occupied by residual graft material. In comparison, the empty groups only had an average fill of 5%, represented by new bone primarily along the defect margins. In summary, bioactive glass putty as a new form of bone graft material demonstrated an ability to promote more rapid early bone formation regeneration due to the resorption of the binder materials, when compared with a bioactive glass particulate, a currently available device.

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