Radiographic and Histologic Comparison of Two Bioactive Glass Bone Void Fillers in a Rabbit Spinal Fusion Model

James F. Kirk¹, Gregg Ritter¹, Michael J. Larson², Robert C. Waters¹, Isaac Finger¹, John Waters¹, Dhyana Sankar¹, James D. Talton¹, Ronald R. Cobb¹,*

¹Research and Development Department, Nanotherapeutics Inc., Alachua, FL
²Ibex Preclinical Research, Logan, UT

Email address: rcobb@nanotherapeutics.com (R. R. Cobb)

To cite this article:

Abstract: Bone graft substitutes and bone graft extenders have been routinely used for spine fusions for decades and have become an essential component in a number of orthopedic applications including spinal fusion. Bioactive glass ceramics have the ability to directly bind to bones and have been widely used as bone graft substitutes due to their high osteoconductivity and biocompatibility. The objective of this study was to compare the fusion rates of two bioactive glass containing bone void fillers (Nano FUSE® and Nova Bone Putty) in a posterolateral fusion rabbit model. Nova Bone Putty and Nano FUSE® alone and in combination with autograft were implanted in the posterior lateral intertransverse process region of the rabbit spine. The spines were evaluated for fusion of the L4-L5 transverse processes in skeletally mature rabbits. Radiographic and histological measurements demonstrated the ability of Nano FUSE® to induce new bridging bone across the transverse processes. The material in combination with autograft performed much better than the material alone. In contrast, Nova Bone Putty did not induce bridging bone across the transverse processes at any time point. This in vivo study demonstrates the novel formulation of Nano FUSE®, a bioactive glass combination with porcine gelatin, could be an effective bone graft extender in posterolateral spinal fusions.

Keywords: Bioactive Glass, Spinal Fusion, Radiography, Histology

1. Introduction

Orthopedic and spine surgeons involved with skeletal repair, reconstruction, and oncology frequently encounter bone defects, and generally achieve satisfactory clinical outcomes with the use of autograft or allograft materials. Although grafting with autogenous iliac bone graft is one of the most commonly performed surgical methods used to create successful bone fusion, various types of artificial bone graft extenders have also been developed due to donor-site morbidity and supply problems [1-6]. Bone graft substitutes offer a wide range of materials, structures, and delivery systems to be used in bone grafting procedures. Synthetic bone graft extenders have been widely used as they pose little risk of disease transmission, are non-immunogenic, and can be produced in large quantities. These materials should possess one or more of the characteristics typical of autograft material including osteoconductivity, osteoinductivity, and osteogenicity. Trials to achieve spinal fusion in animal studies have been made with several bone graft materials including Bioglass, demineralized bone matrix, coraline calcium carbonate, hydroxyapatite, tricalcium phosphate, deep frozen allogeneic bone and autograft bone [7-15].

An ideal bone graft substitute material would facilitate cell adhesion, be tissue compatible, and biodegradable. Since bone is a hard tissue, the bone grafting material should also be able to be replaced by new bone and withstand the rigorous structural forces to which the bone is subjected. As the porosity of the grafting material increases, new bone formation also increases, but the strength decreases [16]. In recent years, newer materials have been developed that have demonstrated improved material strength and toughness while facilitating cell adhesion, growth, and proliferation [17-19].

During the last couple of decades, the development of new implant technologies have shifted from attempts to create a
passive interface between the implant and the native tissue to the design of bioactive materials. Among available alternatives, silica-containing polymers, ceramics, and composites have been reported to show evidence of osteoconduction and biocompatible resorption [20]. Within this category are a wide range of calcium-phosphate ceramics, bioactive glass and bioactive glass-ceramics [21, 22]. Ceramic bone graft extenders account for the largest segment of synthetic of the artificial bone graft extender market. Bioactive glasses are surface-active bone graft materials, which have shown both good biocompatibility and good osteoconduction. The reactivity of the glass is based on the formation of a hydroxyapatite layer on the glass surface, to which bone can chemically bind [23-26]. Bioactive glass ceramics are known to provide a favorable osteoblastic differentiation for mesenchymal stem cells, and can be used as a coating material to enhance resorption and integration between the implant and bone material [25-30]. When in contact with surface-reactive bioactive glass, osteoblasts undergo rapid proliferation forming new bone in roughly the same time period as the normal healing process. In addition, only a minimal amount of bioactive glass is required to induce graft bioactivity. Bioactive glass has been suggested as an alternative for bone replacement and augmentation [31, 32], and has been used in various procedures involving oral surgery, orbit and facial skeleton repair, and calvarial defects [20, 33-35]. It has also been tested in a rabbit model for filling a non-weight bearing defect [29, 36-38]. Based on these properties of bioactive glass, NanoFUSE® was created to take advantage of osteoconductive and proangiogenic properties of bioactive glass. The bioactive glass portion of NanoFUSE® is composed of 45S5 composition disclosed by Hench (also known as Bioglass®).

The present study compares two novel bioactive glass bone void fillers (NovaBone Putty and NanoFUSE®) in a pre-clinical rabbit posterolateral spinal fusion model. In addition, the NanoFUSE® with and without autograft material was compared to autograft material alone in this rabbit model. This animal model has been widely used for evaluating bone void filler materials for spinal fusion. The data presented herein demonstrates that the novel NanoFUSE® formulation of bioactive resulted in more observed spinal fusions than the NovaBone Putty. In addition, the data supports the use of NanoFUSE® as a bone graft extender and provides a basis for its use in clinical applications.

2. Materials and Methods

2.1. Bioactive Glass

Bioactive glass, of the 45S5 composition, was purchased from Mo-Sci Health Care, LLC (Rolla, MO). The composition of the 45S5 (w/w %) was 43 – 47% SiO2; 22.5 – 26.5% CaO; 5 – 7% P2O5; and 22.5 – 26.5% Na2O with a particle size distribution of 90 – 710 µm (≥ 90%). The material was hydrated and warmed immediately prior to implantation. NovaBone Putty, a synthetic device consisting of bioactive glass (calcium phosphosilicate) and a gel-like binder containing glycercin and polyethylene glycol [39], was obtained and prepared using aseptic techniques. NanoFUSE® is a proprietary formulation of bioactive glass and gelatin. Autograft was harvested in select animals from the iliac crests and morselized with Rongeur forceps to an approximate diameter of 5 mm or less. The target volume of bone graft material to be placed for each lateral side of the motion segment was 3cc.

2.2. Surgical Procedures

New Zealand White rabbits were obtained from Western Oregon Rabbit Company (Philomath, OR) at approximately 4 kg. Animals were acclimated to the facility for a minimum of 1 week and completed a pre-study physical examination prior to research use. Rabbits weighed approximately 4 kg at the time of surgery. Each rabbit was weighed prior to surgery to enable accurate calculation of anesthesia drug dosages and to provide baseline body weight for subsequent general health monitoring. Glycopyrrolate (0.1 mg/kg) was administered intramuscularly (IM) approximately 15 minutes prior to anesthesia induction to protect cardiac function during general anesthesia. Butorphanol (1.0 mg/kg) and acepromazine (1-2 mg) were also administered for sedation and early post operative analgesia. General anesthesia was induced with an IM injection of ketamine (25-30 mg/kg) and xylazine (7-9 mg/kg), followed by endotracheal intubation. Anesthesia was maintained with isoflurane (0-4%, to effect) in oxygen. A 24 gauge intravenous (IV) catheter was introduced into the marginal ear vein and secured to the skin. Yohimbine (Yobine, Lloyd Laboratories, Shenandoah, IA), was administered intravenously (0.2 mg/kg) to reverse the adverse cardiovascular effects of xylazine. Cefazolin (30 mg/kg) was administered intravenously for anti-microbial prophylaxis. A fentanyl patch (25 µg/hr) was placed on the skin over the neck for post-operative analgesia. Intra-operative Ringer’s lactate solution was administered intravenously at a rate of 10-20 ml/kg/hr during the surgical procedure.

A dorsoventral radiographic image of the lumbar spine was obtained prior to operative site preparation to identify the targeted L4-L5 operative site. The fur over the operative site was then removed with an electric clipper to expose a sufficient area of skin for aseptic surgery and autograft harvest, if indicated. The skin was subsequently scrubbed with a povidone iodine surgical scrub followed by 70% isopropyl alcohol rinse, repeated three times. Sites were then painted with a povidone iodine solution. The animal was transferred into the operating room and draped for aseptic surgery.

The spine was approached through a single midline skin incision and two paramedian fascial incisions. The L4-L5 levels were identified during surgery by referencing the pre-operative radiographic images and iliac crest palpation. The dorsal surfaces of the transverse processes (TPs) of L4
and L5 were then bilaterally exposed and approximately 2 cm of each TP was decorticated with a motorized burr [40]. Hemorrhage was controlled with pressure and the judicious use of cautery. The gutters were flushed with 1-2 cc of saline to facilitate removal of bone dust and clots. Approximately 3.0 cc of each material was placed in the paraspinal gutters, forming a continuous bridge over and between the decorticated TPs of L4 and L5 (see Table 1 for experimental design). After the bone graft materials were implanted and TP bridging was verified by visual inspection, the fascia was closed with sutures in two layers and the skin was approximated with staples. The rabbits were recovered from anesthesia with supplemental heat and were returned to their home cages after they became ambulatory. Supplemental butorphanol (1 mg/kg) was administered for pain post surgery. Radiographic images were evaluated for bone bridging between the transverse processes of L4-L5 vertebrae. Images that were graded as fused were evaluated for evidence of new bone growth, implant integration, and radiographic fusion, defined as mineralized or trabecular bone bridging between the transverse processes of the L4-L5 lumbar vertebrae. Images that were graded as not fused may have demonstrated considerable new bone in the L4-L5 interspaces, thin radiolucent fissures transversing the fusion masses or radiolucent zones near the vertebrae, interrupting what would otherwise have been a continuous bone bridge between the transverse processes. Images demonstrating significant radiodensity from the implants were graded as “fusion indeterminate.”

2.3. Radiographic Assessment

Posteroanterior radiographs were performed immediately after surgery and at approximately 4, 8, 12, 18, and 24 weeks post surgery. Radiographic images were evaluated for evidence of new bone growth, implant integration, and radiographic fusion, defined as mineralized or trabecular bone bridging between the transverse processes of the L4-L5 lumbar vertebrae. Images that were graded as fused were determined to have a mineralized bone bridge between the L4-L5 vertebrae. Images that were graded as not fused may have demonstrated considerable new bone in the L4-L5 interspaces, thin radiolucent fissures transversing the fusion masses or radiolucent zones near the vertebrae, interrupting what would otherwise have been a continuous bone bridge between the transverse processes. Images demonstrating significant radiodensity from the implants were graded as “fusion indeterminate.”

2.4. Histopathology

Three animals per group were utilized for histological evaluations. Processing of the slides was performed by Laudier Histology (New York, NY). Freshly prepared samples of NanoFUSE® were fixed in 10% formalin, embedded in methyl methacrylate and then sectioned 5µm thick. The sections were stained with toluidine blue to visualize new bone and cartilage formation. Histological scoring was performed based on bilateral assessment as described in Table 2 (A-C). Pathologic evaluation will be performed for the implant sites to determine degree of new bone development in the implant sites as well as to determine spinal fusion (bridging bone).

2.5. Mechanical Testing

All mechanical testing was performed by Numira Biosciences (Bothell, WA). Six samples from each group from the 24-week time point will be properly stored and then evaluated for uniaxial tensile testing. After removal of the remaining muscle and facet joints were removed, pilot holes were drilled ventral to dorsal through two adjacent vertebral bodies. Just prior to testing, the intervertebral disc was divided with a scalpel so that only the intratransverse membrane and fusion mass was left to connect the two adjacent vertebrae. Stainless steel pins were inserted through the pre-drilled holes and connected to a steel wire attached to the material testing device. Biomechanical testing was performed using an Instron 5500R running Bluehill version 2.5 software. Using the jog up controller, each sample was brought to a point where no slack was present in the steel wires hooked to the pins. A tension load was applied to the specimen at a rate of 6mm/min until failure. To obtain maximum load, the cursor was placed at the peak of the load extension curve. To obtain stiffness, the steepest part of the load extension curve was identified and the cursor was placed at the lower end of the slope and then at the upper end. Stiffness was determined as the slope of this line. To obtain energy, if the curve continued to rise without a break or pause in the load-extension curve, the cursor was placed at the point where the curve began to rise and then at the point of the maximum load. If there was a break or pause in the load-extension curve, the cursor was placed at the point where the load-extension curve began to rise and then at the point where the load-extension curve began to pause; then at the point where the pause ends and finally at the point of maximum load. Energy is the area under the curve, which is the sum of two energy values if there is a pause in the curve. Following cursor placement, the software performed the calculations and displayed the results. The software provided Maximum Load, Stiffness, Energy, and Extension (at Maximum Load).

3. Results

3.1. Surgery

Sixty four (64) animals were initially scheduled for surgery, but a total of 63 were operated on for the study (see Table 1 for experimental design). One sham animal died 1 week post-surgery and was not replaced. Weight gain patterns throughout the study term were normal. After several days, surviving rabbits were ambulating normally and demonstrated normal appetites and behavior patterns. These patterns remained normal for the study term.

3.2. Radiographic Analyses

Radiographic images generated for this study were evaluated for evidence of new bone growth, implant integration, and radiographic fusion, defined as mineralized or trabecular bone bridging between the transverse processes of the operated segments. Radiographic fusion was judged by continuous trabecular continuity between L4-L5 transverse processes. Each side was scored independently.
and had to have continuous bridging bone between the transverse processes to be scored as fused (Figures 1 and 2).

At four weeks, the autograft group demonstrated 79% (19/24) fusion while the NanoFUSE® plus autograft demonstrated a 30% (6/20) fusion rate (Table 3). It was also observed that four samples in the NanoFUSE® plus autograft group were observed to contain significant radiodensity from the implant material and were scored as “fusion indeterminate” and not included in the percentage fused scores. NanoFUSE® alone and the sham control did not demonstrate any fusion at this time point. At 8 weeks, the autograft group demonstrated 92% (22/24) fusion rate while the NanoFUSE® plus autograft demonstrated a 38% fusion rate (9/24). By 12 weeks both autograft and NanoFUSE® plus autograft groups demonstrated their maximal fusion rates that were observed at 18 and 24 weeks. The fusion rates for the NanoFUSE® group were roughly half of that observed to autograft alone. This is consistent with the amount of autograft that was loaded into each site. The NanoFUSE® group received half the amount of autograft material per site as the autograft alone sites. NanoFUSE® alone did demonstrate increase spinal fusion rates from 4 weeks to 24 weeks. NanoFUSE® alone demonstrated 4% fusion (1/24) at 12 weeks, 17% (3/18) at 18 and 24 weeks. No fusion was observed at any time point for either the sham or NovaBone Putty treated groups. At the 4 and 8 week time points, the NovaBone Putty demonstrated significant radiodensity from the implant material and these were scored at “fusion indeterminate” and were not included in the overall fusion scores.

3.3. Histological Evaluation

Histologic data are provided in Table 4 and representative images are found in Figure 3 (12 week time point) and Figure 4 (24 week time point). All animals from Group 1 12 week implant sites consisted of variable amounts of new bone with bone marrow, fibrosis and adipose tissue. New bone growth that was observed consisted of a minimal to mild amount of new bone and bone marrow. Two of the implant sites contained a minimal amount of cartilage. In addition, a minimal amount of neovascularization and adipose tissue infiltration was observed. A representative slide from this group at each time point is found in Figures 3A and 4A.

The tissue samples from the autograft group consisted of new bone, bone marrow, fibrosis and adipose tissue at the 12 week time point. Three samples from this group demonstrated 51-100% of bridging of the defect with new bone. The new bone in all of the implant sites consisted of minimum to mild amounts of new bone and a minimal to marked amount of bone marrow. The tissue reaction of these samples contained a minimal number of macrophages and multinucleated giant cells. A representative slide from this group is shown in Figure 3B. The autograft samples from the 24 week group demonstrated very little evidence of the implant material. The samples contained 51-100% of bridging of the defect site with new bone with a moderate amount of bone marrow. A minimal amount of neovascularization was observed in the tissue samples from this group. The tissue reaction of the samples contained a minimal number of macrophages and multinucleated giant cells. A representative slide from this group is presented in Figure 4B.

The NovaBone Putty® implant sites contained a moderate to marked amount of residual implant material at the 12 week time point (76-100%). The implanted material consisted mainly of variably sized clear to pale blue anuclear material. Some of the residual implant material was found within regions of new bone. The percentage of the implant site occupied by new bone was observed to be 1-25%. The new bone consisted of a minimal amount of new bone and bone marrow. The tissue reaction of the implant sites contained a moderate number of macrophages and a minimal number of multinucleated giant cells. The infiltration of adipose tissue that was observed was due to the healing response of the muscle tissue adjacent to the implant sites. A representative slide is presented in Figure 3C. At 24 weeks, all the implant sites contained a moderate amount of residual implant material (51-57%). The implanted material consisted mainly of closely packed pieces of clear to pale blue anuclear material. Some of the pieces were found within new bone. The percentage of the implant site that contained new bone was observed to be 1-25%. The tissue reaction contained a moderate number of macrophages and a mild number of multinucleated giant cells. The minimal amount of adipose that was observed was due to the healing response of the surrounding muscle tissue. A representative slide is presented in Figure 4C.

The NanoFUSE® group samples contained a minimal amount of implanted material (1-25%) at the 12 week time point. The implanted material consisted of variably sized closely packed pieces of clear to pale purple anuclear material. The implant material was found within the new bone growth. There was 26-50% bridging of the defect with new bone and the percentage of the implant site occupied by new bone was 26-50%. The new bone consisted of a minimal to mild amount of new bone and a minimal to moderate amount of bone marrow. The tissue reaction of these sites contained a minimal to moderate number of macrophages and a minimal to mild number of multinucleated giant cells and a minimal amount of adipose tissue. A representative slide is presented in Figure 3D. The NanoFUSE® group samples at the 24 week time point contained a minimal amount (1-25%) of the implanted material. The implanted material consisted of variably sized closely packed pieces of clear to pale purple anuclear material. The implant consisted of a minimal amount of new bone with a minimal to moderate amount of bone marrow and adipose tissue. The implant sites had 1-25% of bridging of the defect with new bone and the percentage of the implant site occupied by new bone was 1-50%. The tissue reaction of all the samples contained a minimal to mild number of macrophages and multinucleated giant cells. A representative slide from this group is presented in Figure 4D.

The NanoFUSE® plus autograft group implants at the 12
week time point contained a minimal amount (1-25%) of the implanted material. The implanted material consisted of small fragments of light blue anuclear material. The implant sites consisted of a mild amount of new bone, a moderate amount of bone marrow and a minimal amount of cartilage. There was 1-25% bridging of the defect with new bone and the percentage of implant sites occupied by new bone was 1-25%. The tissue reaction to these implants contained a minimal to mild amount of adipose tissue, a minimal number of plasma cells, macrophages and multinucleated giant cells. A representative slide is shown in Figure 3E. At the 24 week time point, the NanoFUSE® plus autograft group implant sites contained a minimal to mild amount of new bone and a mild to moderate amount of bone marrow. A minimal amount (1-25%) of the implanted material was still visible as variably sized closely packed pieces of dark blue anuclear material. All of the implant sites in this group had 1-25% bridging of the defect with new bone and the percentage of the implant site occupied by new bone was 1-25%. The new bone consisted of a minimal amount of new bone and bone marrow. There was also a minimal amount of neovascularization observed. The tissue reaction of all the implants contained a mild to moderate number of macrophages and a minimal amount of multinucleated giant cells. A representative slide is presented in Figure 4E.

3.4. Mechanical Testing

During preparation of the specimens, the facet joint (dorsal elements) connecting the vertebrae at the fusion level on one specimen from the NanoFUSE® only group was not removed. Data from this sample reflects the strength of both the fusion mass and the dorsal elements and therefore was removed from the dataset.

The autograft, NanoFUSE® alone, NanoFUSE® plus autograft, and NovaBone Putty appeared to have higher maximum loads than the sham treated group (Table 5). The maximum load was 159.77±44.58 N for the Sham group, 229.32±94.44 N for the autograft group, 198.69±136.65 N for the NovaBone Putty group, 226.92±25.78 N for the NanoFUSE® group and 177.82±58.87 N for the NanoFUSE® +autograft group. However, these differences were not significant. The autograft group appeared to have the highest values for stiffness while the other groups had similar values. The stiffness values were 62.62±16.86 N/mm for the sham group, 90.39±18.39 N/mm for the autograft group, 50.24±28.34 N/mm for the NovaBone Putty group, 57.17±47.19 N/mm for the NanoFUSE® group and 56.47±22.17 N/mm for the NanoFUSE® plus autograft group. However, these differences were not statistically significant.

NanoFUSE® had the highest values based on extension data followed by NovaBone Putty, while the other groups had similar values. The extension values were 6.27±1.38 cm for the sham group, 5.13±2.73 cm for the autograft group, 8.45±3.91 cm for the NovaBone Putty group, 11.23±3.74 cm for the NanoFUSE® group, and 6.45±8.0.98 for the NanoFUSE® plus autograft group. However, these differences were not statistically significant. With the exception of NovaBone Putty, all the groups had similar values for energy. The energy values were 299.33±123.32 mJ for the sham group, 339.21±283.28 mJ for the autograft group, 207±139.33 mJ for the NovaBone Putty group, 332.98±156.85 mJ for the NanoFUSE® group alone, and 366.61±163.04 mJ for the NanoFUSE® plus autograft group. However, these differences were not statistically significant.

4. Discussion

The development of novel bone graft substitutes with novel properties can expand the use of these materials in orthopedic treatments. Bone graft substitutes should possess one or more of the characteristics typical of autograft. These materials should be biocompatible, possess osteoconductive as well as osteoinductive properties, and should degrade in concert with bony replacement. In addition, an ideal bone graft substitute and bone graft extender materials should have the ability to maintain strength and stability of porous structures during resorption until achieving bridging bone, to be replaced with new bone and have adequate resorption and remodeling rates. Many different biomaterials are becoming available for use in orthopedic reconstruction [41, 42]. Bioactive glasses are reported to be osteoconductive and biocompatible [20]. Studies have shown that new bone formation associated with particles of 45S5 Bioglass was significantly greater and more rapid than that of other synthetic materials in a rabbit femoral defect model [36].

Bioactive glasses are a group of silica-based materials used as bone substitutes, typically in the form of powders and rigid monoliths. Bioactive glass is the first man-made material to form a direct chemical bond with bone. It is also the first man-made material to exert a positive effect on osteoblastic differentiation and osteoblast proliferation [43]. Regardless of their specific composition, bioactive glasses form an apatite-like surface layer under physiological conditions, which is a pre-requisite for their bone binding capability [44]. Clinical use of bioactive glass has been limited to the replacement of bony tissues under low loads and as bone void fillers. Years of testing, preclinical, and clinical use have demonstrated the safety and efficacy of this material [45]. Bioactive glass has traditionally been employed for its osteoconductive and osteostimulative properties [43, 46, 47]. Recently, data has been presented demonstrating the proangiogenic potential of bioactive glass in vitro and in vivo [47]. In addition, these studies have shown that the soluble dissolution products of bioactive glass can stimulate the production of proangiogenic factors thereby providing a potentially promising strategy to enhance neovascularization and resultant bone formation.

It is clear from the data presented herein, that the NanoFUSE® formulation samples had increased new bone formation as well as bridging bone relative to the NovaBone Putty®. The radiographic data demonstrated that the NanoFUSE® generated bridging bone while the bioactive glass product, NovaBone Putty®, did not. In addition, the NanoFUSE® formulation of bioactive glass generated more
bone as measured histologically compared to NovaBone Putty®. The addition of autograft material to NanoFUSE® resulted in bridging bone approaching the level as autograft alone. These results demonstrate that NanoFUSE® could be a very effective autograft extender.

Our results are in contrast to those of previous studies that observed bioactive glass implants were associated with an inflammatory reaction [48]. Some authors have suggested that bioactive glass particles may contain cracks and that cells can migrate within these cracks to a central cavity with the bioactive glass particle [38, 49]. Acute inflammation was also observed with bioactive glass implants in a sheep vertebral defect model [50]. NanoFUSE® alone or in combination with autograft did not result in an inflammatory response that was different from the autograft control. The new bone formation observed with NanoFUSE® is consistent with previous studies [39, 51]. Bone formation was observed throughout the defect on the surfaces of particles, bridging between the particles and anchoring them together as a three-dimensional lattice, supporting and stimulating further bone growth.

Similar models have been used to verify autograft extenders with reproducible results. The fusion spinal rate in the autograft control group is consistent with the rate demonstrated in previous studies [40, 52-56]. NanoFUSE® in combination with autograft demonstrated increased fusion rates when compared to sham controls as measured radiographically. The ability of NanoFUSE® to homogeneously mix with the morselized autograft allowed a continuous mixture of substrate with minimal void within the graft site for new bone to develop and fuse the motion segment. Radiographic analyses also showed higher fusion rates with NanoFUSE® implants compared to sham controls. In contrast, no bridging bone was observed in any of the NovaBone Putty implants at any time point. It is possible that bone may have been formed in the 4 and 8 week time points where significant radiodensity was observed from the implant material. This is unlikely due to the observation that fusion was observed in with these implants at later time points.

This study has a few limitations. The rabbit model has been widely used for evaluating spinal surgery technique and spinal fusion implant materials, but as with any animal study, results cannot be directly extrapolated to more advanced, clinical scenarios. The limited number of rabbits per study group may not accurately reflect the range of pathology (age, osteoporosis, trauma) or systemic agents (steroids, smoking, malnutrition) that may be present in a clinical cohort [57]. In addition, there are differences in the multiple modalities used to evaluate fusion. Although histologic analysis is highly sensitive for detecting fusion, individual sections are prone to miss bridging bone that exists beyond the plane sectioned for study. It also should be noted that rabbit DBM was used instead of human DBM and this may not function in an identical fashion as human DBM.

The results of this rabbit spinal fusion study demonstrate the biocompatibility of the NanoFUSE® material. It also demonstrates that the NanoFUSE® material is significantly resorbed (only 1-25% of the implanted material being observed) and replaced with new bone within 24 weeks. In contrast, greater than 75% of the implant site was occupied by residual NovaBone Putty at the 12 week time point and greater than 50% of the implant site was occupied by NanoBone Putty at the 24 week time point. Similar levels of cellular influxes were observed in the NanoFUSE® plus autograft group compared to the autograft group. NovaBone Putty implant sites were marked by higher levels of macrophages than the other groups. New bridging bone was also observed in animals implanted with NanoFUSE® and NanoFUSE® plus autograft. The results also suggest that NanoFUSE® is effective in producing a posterolateral fusion by radiographic criteria in an extender mode. This study demonstrates radiographically and histologically the ability of NanoFUSE® to support new bone formation and bridging fusion in the rabbit spinal fusion model, especially when used as an autograft extender. Similarly, biomechanical data showed comparable values for load, stiffness, extension and energy between NanoFUSE® plus autograft and autograft alone. While animal models cannot be translated into clinically successful human applications, the results of this study suggest that further investigation into the clinical use of this material as a standalone bone void filler or as a graft extender is warranted. NanoFUSE® is a registered trademark of Nanotherapeutics, Inc.

**Acknowledgements**

The authors would also like to thank Nanotherapeutics, Inc. for their continued support of this research.

<table>
<thead>
<tr>
<th>Group Identification</th>
<th>Implant volume (cc/side)</th>
<th>Number of Animals per Duration</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>1 Day</td>
</tr>
<tr>
<td>1</td>
<td>Sham defect</td>
<td>0</td>
</tr>
<tr>
<td>2</td>
<td>Autograft (Positive Control)</td>
<td>3</td>
</tr>
<tr>
<td>3</td>
<td>NanoBone Putty®</td>
<td>3</td>
</tr>
<tr>
<td>4</td>
<td>NanoFUSE® bone void filler</td>
<td>3</td>
</tr>
<tr>
<td>5</td>
<td>NanoFUSE® bone void filler with autograft</td>
<td>1.5 bone filler + 1.5 autograft</td>
</tr>
</tbody>
</table>
Table 2. Histological Scoring Criteria.

<table>
<thead>
<tr>
<th>Inflammation</th>
<th>Score</th>
<th>0 = Minimal</th>
<th>1 = Mild</th>
<th>2 = Moderate</th>
<th>3 = Marked</th>
</tr>
</thead>
<tbody>
<tr>
<td>Heterophils</td>
<td></td>
<td>Rare, 1-5/HPF</td>
<td>6-10/HPF</td>
<td>Heavy Infiltrate</td>
<td>Packed</td>
</tr>
<tr>
<td>Lymphocytes</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Plasma Cells</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Eosinophils</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Macrophages</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Multinucleated Giant Cells</td>
<td></td>
<td>Rare, 1-2/HPF</td>
<td>3-5/HPF</td>
<td>Heavy Infiltrate</td>
<td>Sheets</td>
</tr>
<tr>
<td>Necrosis *</td>
<td></td>
<td>Minimal</td>
<td>Mild</td>
<td>Moderate</td>
<td>Marked/Severe</td>
</tr>
</tbody>
</table>

HPF=high powered field (400x), averaged over the entire implant site

B. Severity Scale for Regenerative and Degenerative Tissue Responses:

1 = Minimal / Slight, approximately 1 – 25% of the tissue reaction was involved
2 = Mild, approximately 26 – 50% of the tissue reaction was involved
3 = Moderate, approximately 51 – 75% of the tissue reaction was involved
4 = Marked / Severe, approximately 76 – 100% of the tissue reaction was involved
NA = Not applicable. Material was not implanted into the surgical site.

C. Severity Scale for Percentages of Tissue Response Score:

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Definition</th>
<th>Score</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bridging (% of the original defect bridged by new bone)</td>
<td>100% bridging across the defect</td>
<td>4</td>
</tr>
<tr>
<td></td>
<td>51% - 99% bridging across the defect</td>
<td>3</td>
</tr>
<tr>
<td></td>
<td>26% - 50% bridging across the defect</td>
<td>2</td>
</tr>
<tr>
<td></td>
<td>1% - 25% bridging across the defect</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>0% bridging across the defect</td>
<td>0</td>
</tr>
<tr>
<td>Amount of New Bone (% of the defect area occupied by new bone)</td>
<td>76% - 100% new bone formation in defect area</td>
<td>4</td>
</tr>
<tr>
<td></td>
<td>51% - 75% new bone formation in defect area</td>
<td>3</td>
</tr>
<tr>
<td></td>
<td>26% - 50% new bone formation in defect area</td>
<td>2</td>
</tr>
<tr>
<td></td>
<td>1% - 25% new bone formation in defect area</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>0% new bone formation in defect area</td>
<td>0</td>
</tr>
<tr>
<td>Fibrosis (% of the defect area occupied by fibrous connective tissue)</td>
<td>76% - 100% fibrosis in defect area</td>
<td>4</td>
</tr>
<tr>
<td></td>
<td>51% - 75% fibrosis in defect area</td>
<td>3</td>
</tr>
<tr>
<td></td>
<td>26% - 50% fibrosis in defect area</td>
<td>2</td>
</tr>
<tr>
<td></td>
<td>1% - 25% fibrosis in defect area</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>0% fibrosis in defect area</td>
<td>0</td>
</tr>
<tr>
<td>Inflammation (% of the defect area occupied by inflammatory cells)</td>
<td>76% - 100% inflammation in defect area</td>
<td>4</td>
</tr>
<tr>
<td></td>
<td>51% - 75% inflammation in defect area</td>
<td>3</td>
</tr>
<tr>
<td></td>
<td>26% - 50% inflammation in defect area</td>
<td>2</td>
</tr>
<tr>
<td></td>
<td>1% - 25% inflammation in defect area</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>0% inflammation in defect area</td>
<td>0</td>
</tr>
<tr>
<td>Remnant Implant Material (% of the defect area occupied by residual implant material)</td>
<td>76% - 100% remnant implant material in the defect area</td>
<td>4</td>
</tr>
<tr>
<td></td>
<td>51% - 75% remnant implant material in the defect area</td>
<td>3</td>
</tr>
<tr>
<td></td>
<td>26% - 50% remnant implant material in the defect area</td>
<td>2</td>
</tr>
<tr>
<td></td>
<td>1% - 25% remnant implant material in the defect area</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>0% remnant implant material in the defect area</td>
<td>0</td>
</tr>
</tbody>
</table>

Table 3. Radiographic Fusion Results.

<table>
<thead>
<tr>
<th>Group</th>
<th>Test Article</th>
<th>Radiographic Fusion Results per Time Point (Total Sites Fused/Total Sites % Fused)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>4 Weeks</td>
</tr>
<tr>
<td>1</td>
<td>Sham defect</td>
<td>0/22 0%</td>
</tr>
<tr>
<td>2</td>
<td>Autograft (Positive Control)</td>
<td>19/24 79%</td>
</tr>
<tr>
<td>3</td>
<td>NovaBone Putty</td>
<td>24 segments ‘fusion indeterminate’</td>
</tr>
<tr>
<td>4</td>
<td>NanoFUSE® bone void filler</td>
<td>0/24 0%</td>
</tr>
<tr>
<td>5</td>
<td>NanoFUSE® bone void filler with autograft</td>
<td>6/20 30%</td>
</tr>
</tbody>
</table>

Segments that were graded as ‘fusion indeterminate’ were not included in the percent fused.
### Table 4. Comparison of the Averages for the Groups 1-4 Implant Sites.

#### A. 12 Week Sites

<table>
<thead>
<tr>
<th>Group</th>
<th>Implant Material</th>
<th>1 Sham</th>
<th>2 Autograft (Positive Control)</th>
<th>3 NovaBone Putty®</th>
<th>4 NanoFUSE®</th>
<th>5 NanoFUSE® with Autograft</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>INFLAMMATION</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Heterophils (Neutrophils)</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>Lymphocytes</td>
<td>0</td>
<td>1</td>
<td>0</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>Plasma Cells</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>Macrophages</td>
<td>1</td>
<td>1</td>
<td>3</td>
<td>2</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>Multinucleated Giant Cells</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>TOTAL INFLAMMATION SCORE</td>
<td>2</td>
<td>3</td>
<td>4</td>
<td>4</td>
<td></td>
</tr>
<tr>
<td></td>
<td>REGENERATIVE TISSUE RESPONSE (R)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>New bone</td>
<td>2</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>New bone marrow</td>
<td>2</td>
<td>2</td>
<td>1</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>New cartilage</td>
<td>1</td>
<td>0</td>
<td>1</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>Neovascularization</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>Myofiber regeneration</td>
<td>1</td>
<td>0</td>
<td>1</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>REGENERATIVE TISSUE RESPONSE SCORE (R)</td>
<td>7</td>
<td>4</td>
<td>5</td>
<td>3</td>
<td>4</td>
</tr>
<tr>
<td></td>
<td>DEGENERATIVE TISSUE RESPONSE (D)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Adipose tissue infiltration</td>
<td>2</td>
<td>1</td>
<td>1</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td></td>
<td>Hemorrhage</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>Myofiber degeneration and/or necrosis</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>DEGENERATIVE TISSUE RESPONSE SCORE (D)</td>
<td>3</td>
<td>2</td>
<td>2</td>
<td>3</td>
<td>3</td>
</tr>
<tr>
<td></td>
<td>OVERALL TISSUE RESPONSE SCORE (R-D)</td>
<td>4</td>
<td>2</td>
<td>3</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>% Of bridging of the original defect by new bone</td>
<td>2</td>
<td>3</td>
<td>1</td>
<td>2</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>% Of the defect area occupied by new bone</td>
<td>2</td>
<td>3</td>
<td>1</td>
<td>2</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>% Of defect area occupied by fibrous connective tissue</td>
<td>2</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>% Of defect area occupied by inflammatory cells</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>% Of defect area occupied by residual implant material</td>
<td>NA</td>
<td>0</td>
<td>4</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>OVERALL TISSUE RESPONSE SCORE (R-D)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>% Of bridging of the original defect by new bone</td>
<td>1</td>
<td>3</td>
<td>1</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>% Of the defect area occupied by new bone</td>
<td>1</td>
<td>3</td>
<td>1</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>% Of defect area occupied by fibrous connective tissue</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>% Of defect area occupied by inflammatory cells</td>
<td>0</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>% Of defect area occupied by residual implant material</td>
<td>NA</td>
<td>1</td>
<td>3</td>
<td>1</td>
<td>1</td>
</tr>
</tbody>
</table>

#### B. 24 Week Sites

<table>
<thead>
<tr>
<th>Group</th>
<th>Implant Material</th>
<th>1 Sham</th>
<th>2 Autograft (Positive Control)</th>
<th>3 NovaBone Putty®</th>
<th>4 NanoFUSE®</th>
<th>5 NanoFUSE® with Autograft</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>INFLAMMATION</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Heterophils (Neutrophils)</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>Lymphocytes</td>
<td>0</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>Plasma Cells</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>Macrophages</td>
<td>0</td>
<td>1</td>
<td>3</td>
<td>2</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>Multinucleated Giant Cells</td>
<td>0</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>TOTAL INFLAMMATION SCORE</td>
<td>0</td>
<td>3</td>
<td>4</td>
<td>3</td>
<td>4</td>
</tr>
<tr>
<td></td>
<td>REGENERATIVE TISSUE RESPONSE (R)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>New bone</td>
<td>1</td>
<td>2</td>
<td>1</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>New bone marrow</td>
<td>1</td>
<td>3</td>
<td>1</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>New cartilage</td>
<td>0</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>Neovascularization</td>
<td>0</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>Myofiber regeneration</td>
<td>0</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>REGENERATIVE TISSUE RESPONSE SCORE (R)</td>
<td>2</td>
<td>8</td>
<td>3</td>
<td>3</td>
<td>3</td>
</tr>
<tr>
<td></td>
<td>DEGENERATIVE TISSUE RESPONSE (D)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Adipose tissue infiltration</td>
<td>4</td>
<td>1</td>
<td>1</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td></td>
<td>Hemorrhage</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>Myofiber degeneration and/or necrosis</td>
<td>1</td>
<td>0</td>
<td>1</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>DEGENERATIVE TISSUE RESPONSE SCORE (D)</td>
<td>5</td>
<td>1</td>
<td>2</td>
<td>3</td>
<td>3</td>
</tr>
<tr>
<td></td>
<td>OVERALL TISSUE RESPONSE SCORE (R-D)</td>
<td>-3</td>
<td>7</td>
<td>1</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>% Of bridging of the original defect by new bone</td>
<td>1</td>
<td>3</td>
<td>1</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>% Of the defect area occupied by new bone</td>
<td>1</td>
<td>3</td>
<td>1</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>% Of defect area occupied by fibrous connective tissue</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>% Of defect area occupied by inflammatory cells</td>
<td>0</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>% Of defect area occupied by residual implant material</td>
<td>NA</td>
<td>1</td>
<td>3</td>
<td>1</td>
<td>1</td>
</tr>
</tbody>
</table>

NI= Not Included
Table 5. Analysis of Mechanical Data.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Load (N)</th>
<th>Stiffness (N/mm)</th>
<th>Extension (cm)</th>
<th>Energy (mJ)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sham Control</td>
<td>159.77±44.58</td>
<td>62.15±18.86</td>
<td>6.27±1.38</td>
<td>294.83±114.89</td>
</tr>
<tr>
<td>Autograft</td>
<td>229.32±94.44</td>
<td>90.39±18.39</td>
<td>5.13±2.73</td>
<td>339.21±283.28</td>
</tr>
<tr>
<td>NovaBone Putty®</td>
<td>198.69±136.66</td>
<td>50.24±28.34</td>
<td>8.46±3.91</td>
<td>207.21±139.33</td>
</tr>
<tr>
<td>NanoFUSE®</td>
<td>226.92±25.70</td>
<td>57.17±27.19</td>
<td>11.23±3.74</td>
<td>337.58±27.98</td>
</tr>
<tr>
<td>NanoFUSE®+autograft</td>
<td>177.82±58.87</td>
<td>56.47±22.17</td>
<td>6.45±0.98</td>
<td>514.81±252.32</td>
</tr>
</tbody>
</table>

Figure 1 Representative radiographs of spines from 12-week samples. Implant site is identified by the staple in the radiographs. (A) sham; (B) autograft; (C) NovaBone Putty®; (D) NanoFUSE®; (E) NanoFUSE®+autograft.

Figure 2 Representative radiographs of spines from 24-week samples. Implant site is identified by the staple in the radiographs. (A) sham; (B) autograft; (C) NovaBone Putty®; (D) Rabbit NanoFUSE® DBM; (E) Rabbit NanoFUSE® DBM+autograft.

Figure 3 Representative histological slides of spines from 12-week samples. Freshly prepared samples were fixed in 10% formalin, embedded in methyl methacrylate and then sectioned 5µm thick. The sections were stained with toluidine blue. (A) Representative slide of a Group 1 (Sham Defect) 12 week implant site – whole implant site photo at 20x magnification.; (B) Representative slide of a Group 2 (Autograft (Positive Control)) 12 week implant site – whole implant site photo at 20x magnification. (C) Representative slide of a NovaBone Putty® 12 week implant site – whole implant site photo at 20x magnification. (D) Representative slide of a NanoFUSE® 12 week implant site – whole implant site photo at 20x magnification. (E) Representative slide of NanoFUSE® with Autograft 12 week implant site – whole implant site photo at 20x magnification.

Figure 4 Representative histological slides of spines from 24-week samples. Freshly prepared samples were fixed in 10% formalin, embedded in methyl methacrylate and then sectioned 5µm thick. The sections were stained with toluidine blue. (A) sham; (B) Representative slide of an Autograft Positive Control 24 week implant site – whole implant site photo at 20x magnification. (C) Representative slide of a NovaBone Putty® 24 week implant site – whole implant site photo at 20x magnification (D) Representative slide of a NanoFUSE® 24 week implant site – whole implant site photo at 20x magnification. (E) Representative slide of NanoFUSE® with Autograft 24 week implant site – whole implant site photo at 20x magnification.

A. Sham Group
B. Autograft

C. NovaBone Putty®
D. NanoFUSE®

![Image](image1.jpg)

E. NanoFUSE® + Autograft

![Image](image2.jpg)

Figure 1. 12 week samples.
A. Sham Group

B. Autograft
C. NovaBone Putty®

D. NanoFUSE®
E. NanoFUSE® + Autograft

Figure 2. 24 week samples.

A. Sham Defect
B. Autograft Material

![Autograft Material Image]

C. NovaBone Putty®

![NovaBone Putty® Image]

D. NanoFUSE®

![NanoFUSE® Image]
E. NanoFUSE® plus Autograft

**Figure 3.** Histology Results from Twelve Week Samples.

A. Sham Defect

B. Autograft
C. NovaBone Putty

D. NanoFUSE®

E. NanoFUSE® plus Autograft

Figure 4. Histology Results from Twenty-four Week Samples.
References


