

Comparative Histologic and Calcium Content Evaluation of Osteofil™, Grafton™ and Dynagraft™ Putty Bone Inductive Materials in the Nude Rat at 28 Days

COMPARATIVE HISTOLOGIC AND CALCIUM CONTENT EVALUATION OF OSTEOFIL™, GRAFTON™ AND DYNAGRAFT™ PUTTY BONE INDUCTIVE MATERIALS IN THE NUDE RAT AT 28 DAYS

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Purpose: The objective of this study was to assess the osteoinductivity of three commercially available demineralized allograft composites by histologic and radiographic evaluation of de novo bone formed as a consequence of intramuscular implantation in athymic nude rats.

Material and Methods: Test and control articles were obtained from materials sold to hospitals in the United States. Test articles consisted of Grafton™ Putty (Osteotech, Inc.), Osteofil™ Putty (Regeneration Technologies, Inc.) and Dynagraft™ Putty (GenSci, Inc.). One cc of each test material was obtained from three different lots. The density of each test material was measured and used to weigh 0.04 cc of test material for implantation. Twenty-six nude athymic rats were used as the test species. Six implants were placed in rectus abdominus muscle pouches in each animal. Twenty-three animals went to term with 137 explanted specimens available for evaluation. Histological analysis and scoring for new bone growth was carried out in 69 explants and atomic absorption spectroscopy analysis for calcium content was carried out in 68 explants. The size of the residual matrix as a fraction of the original implant volume was calculated. The matrix was evaluated histologically and scored for percent matrix volume occupied by bone (bone induction response score) on a 4 point scale (4~100%, 3~75%, 2~50%, 1~25% and 0~0%). The product of the residual matrix volume and percent matrix volume occupied by bone was recorded to reflect total bone volume as a fraction of the original implant volume. This was calculated for each of the implants.

Results: All Grafton™ and Osteofil™ specimens showed evidence of bone induction. None of the Dynagraft™ specimens showed any evidence of bone or cartilage formation. Implants with bone formation had residual matrix ranging from 10 to 100 percent. Grafton™ had a mean residual matrix of 40.5% and Osteofil™ 77.3%.

The mean bone induction response score (% of matrix occupied by bone) for Grafton™ was 3.52, Osteofil™ was 2.27 and Dynagraft™ was 0.0. The interpreted total bone volume (residual matrix volume X % matrix volume occupied by bone) for Grafton™ was 36% for Osteofil™ 44% and for Dynagraft™ 0%.

Dynagraft™ displayed the most consistent and significant pathologic response. The adverse response was characterized by chronic inflammation, foreign body giant cell response and fibrous connective tissue throughout the implant.

All implants showing bone formation were stratified with bone formation in the central specimen and no bone induction and chronic inflammation at the implant periphery. Radiographic films taken ex vivo at term were reviewed and compared among treatments. All were irregular in shape, varied in density, size and presence of granular structures. Radiographically, the Grafton™ implanted sites had the smallest ossicle, Dynagraft™ the largest and Osteofil™ in the middle. Grafton™ explants were significantly ($p < 0.05$) less dense, and had fewer x-ray dense granular structures than the other two test materials. Dynagraft™ explants contained the most calcium.

Discussion: The results of this study clearly indicate that Grafton™ and Osteofil™ implants induced bone but not Dynagraft™. The total volume of new bone formed was not significantly different between Grafton™ and Osteofil™ explants. Chronic inflammation was present throughout the Dynagraft™ explant. The increased calcification in the Dynagraft™ explant was possibly due an increased inflammatory response as well as calcification of the polymer.

DOSE DEPENDENT TOXICITY OF A COMMERCIALLY AVAILABLE DEMINERALIZED BONE MATRIX MATERIAL

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Introduction: The safety of a commercially available demineralized bone matrix derived from human tissue was recently questioned after high doses induced rat fatalities. Grafton demineralized bone matrix (DBM) contains a significant percentage of glycerol. The toxic effects of glycerol have been well documented in prior studies as leading to acute renal failure. The safety of higher doses of this glycerol-containing substance has not been reported. A prospective evaluation of the toxic effects of two commercially available grafton demineralized bone matrix products was performed in an athymic rat. The purpose of this study was to evaluate the possible dose dependent adverse effects of two commercially available demineralized bone matrix (Grafton putty - Osteotech Inc. NJ and Osteofil allograft bone paste - Regeneration Technologies, FL).

Methods: Phase I: Fourteen male athymic nude rats, 330 to 550g in weight, were implanted with three different doses of commercially available demineralized bone matrix 'grafton putty' in their hindlimbs. The putty was placed between the muscles of the hindlimb adjacent to the femur. Rats were observed for adverse effects and early death. The tissues were preserved.

Phase II: Fifteen female athymic nude rats, 190 to 230g in weight, were randomly implanted with any of three different doses of grafton putty or two different doses of Osteofil in their hindlimbs. One rat received a high dose grafton implant on both sides of the spine.

Results: *Phase I:* All of the rats (100%) implanted with the highest dose of putty (0.008cc/gram) died within twelve hours of implantation (5/5 rats). One of the four rats with the intermediate dose (0.004cc/gram) died within twelve hours of implantation. By seventy-two hours after implantation, three of the four animals (75%) with the intermediate dose (0.004cc/gram) had died. All of the animals receiving the lowest dose (0.002cc/gram) survived to two weeks time (4/4 rats). Histological analysis of the rat tissues reveals the cause of death to be associated with acute tubular necrosis. *Phase II:* All of the rats implanted in the hindlimb with the highest dose of grafton putty (0.008cc/gram) died within 4 days of implantation (3/3 rats). One rat implanted with the high dose of grafton in the paraspinal muscles survived without any signs of adverse effects. All of the grafton medium dose (2/2 rats) (0.004cc/gram) and low dose (2/2) (0.002cc/gram) implanted rats survived until sacrifice. One rat implanted with a high dose of Osteofil died in 72 hours. All of the remaining Osteofil-implanted rats survived until sacrifice.

Conclusions: Commercially available grafton putty leads to death in athymic rats in a dose-dependent manner. A potential source of this toxicity is the glycerol contained in the material. The lowest dose in this study, which did not demonstrate any adverse effects, is still higher than the typical dose used in human subjects. One animal receiving a high dose of Osteofil also died, although this product did not induce death in a dose-dependent manner. Although this is a preliminary study, these results raise concerns for possible adverse effects in humans with high doses of Grafton. Because of the widespread use of this product clinically in patients with unknown tolerance levels, the authors feel compelled to report these findings. Further study regarding the safety of this product, the specific tolerances and effective doses in humans is certainly warranted.

AN UNEXPECTED OUTCOME DURING TESTING OF COMMERCIALY AVAILABLE DEMINERALIZED BONE GRAFT MATERIALS

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Purpose: Over the last decade, several different commercially available processed demineralized bone matrix grafting materials have become available for human use. However, there have been no side-by-side comparison of “off the shelf” versions of these products with regard to their ability to form bone in vivo. The purpose of this study was to perform this comparison with commercially available materials in an established animal model.

Material and Methods: Six different types of bone graft materials obtained from our operating room inventory were placed subcutaneously and intermuscularly in athymic homozygous rats. The bone graft volume used was 1 cc at each site. At the time of sacrifice, the newly formed bone nodules were dissected from the surrounding tissue and high resolution faxitron radiographs were obtained. Samples were analysed for undemineralized histomorphometric analysis. The internal organs of the animals were also removed for pathological analysis.

Results: During the initial two post operative days 8 of the 19 animals on died. All animals that died had received Grafton™ Gel or Putty. Of the 9 animals receiving the Grafton™ materials (either Gel or Putty), 8 died ($p < 0.001$). The mean dose of the Grafton™ material was 8.6 ± 0.7 cc/kg. The animals whose deaths were observed demonstrated hematuria prior to expiring.

The high mortality rates prompted the initial experiment to be halted. The protocol was reviewed and revised to implant 0.25 cc at each site instead of the original 1 cc. The remaining 11 animals were implanted with the lower volume. Of this second group, 2 of the 6 animals receiving the Grafton™ materials expired with hematuria prior to death. The mean dose of Grafton™ implanted in this second experiment was 1.6 ± 0.2 cc/kg.

None of the animals implanted with any other bone matrix product (Opteform™, Osteofil™, Dynagraft™ Gel and Putty) died. Three different lots of Grafton™ Gel and Putty, manufactured on different days, were used. Histologic analysis of the kidneys in all the rats that died demonstrated evidence of acute tubular necrosis with some glomerular hemorrhage. These findings were consistent with an acute toxic reaction.

Discussion: The results after the use of Grafton™ in this study were unexpected. While the exact cause of death remains unclear, histologic analysis appears to implicate an acute toxic reaction causing acute tubular necrosis and subsequent death. It appears that the glycerol in the Grafton™ may be responsible. Glycerol has been implicated in renal toxicity and there is a well established glycerol-induced acute renal failure model in the rat. Although the results of this preliminary investigation may have no clinical implication in healthy patients implanted with lower doses of Grafton™, we feel that glycerol containing bone matrix products must be used with extreme caution in lower weight pediatric patients and those at risk for renal disease.

Prospective Comparison of Commercially Available Demineralized Bone Matrix for Spinal Fusion

PROSPECTIVE COMPARISON OF COMERCIAALLY AVAILABLE DEMINERALIZED BONE MATRIX FOR SPINAL FUSION

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Introduction: Demineralized bone matrix derived from human tissues has demonstrated the ability to aid in the stimulation of an osteoinductive response allowing for improved bone growth and fusion. In an effort to augment the available grafting material as well as increase fusion rates, the utilization of a demineralized bone matrix (DBM) as a graft extender or even as a graft substitute has become commonplace. A variety of human DBMs are commercially available, but no comparative studies exist examining their relative effectiveness in spinal fusion. The athymic rat offers a unique animal model with the advantage of a non-immunogenic environment for in vivo evaluation of fusion. The purpose of this study was to compare the osteoinductive ability between three commercially available human DBMs to heal a spinal fusion.

Methods: Fifty-eight mature athymic nude female rats were used in this study (175-240 g, Harlan Sprague Dawley, IN). Three groups of 18 rats each were implanted with one of the three DBMs evaluated (Dynagraft putty - Gen-Sci, Regeneration Laboratories, CA, Grafton putty - Osteotech Inc. NJ, and Osteofil allograft bone paste - Regeneration Technologies, FL), and the rats were sacrificed at 2, 4, 6, and 8 weeks. The remaining four rats received human cortical bone graft and were sacrificed at 6 weeks. All procedures were performed by a single surgeon through a midline incision, exposing the transverse processes. Decortication was performed on the L4 and L5 (lamina and facet joints were left intact without decortication). An aliquot equal to 0.3 cc as measured in a 1.0 cc syringe was placed into each posterolateral gutter spanning the transverse processes for a total of 0.6 cc of graft per animal. The wound was irrigated and closed. High Resolution Radiographs were obtained in vivo and after sacrifice. Explanted lumbar spines were manually tested for intersegmental motion by three independent observers blinded to the treatments. Any motion detected for either side between facets or between transverse processes of L4 and L5 by manual testing was considered a failure of fusion. Absence of any motion was considered successful fusion. Histological analysis consisted of non-decalcified sections.

Results: Overall, a majority of the L4-L5 levels implanted with Osteofil or Grafton fused compared to none of spines implanted with Dynagraft (comparison at 6 weeks, $P < 0.0001$, Fischer's Exact Test*). Even after 8 weeks, Dynagraft did not restrict motion and no bone formation was noted radiographically.

All spines considered fused contained a large fusion mass, whereas the spines implanted with Dynagraft, containing an equally abundant bulk as the others (Grafton and Osteofil), were easily manually flexed, and the material was minimally incorporated, even at 8 weeks. These data support the athymic rat as a model for effective evaluation of the relative efficacy of these as well as other human DBMs to induce posterolateral intertransverse process fusion. Control rats receiving autogenous iliac crest bone taken from a separate experiment did not result in spinal fusion. Non-decalcified histology confirmed the presence of a pseudarthrosis and the presence of a solid fusion, and the histology correlated with the manual testing.

Conclusions: This is the first highly controlled, prospective testing of three commercially available demineralized bone matrix products in a spinal fusion model. The athymic nude rat model allows for each substance to be used in its commercial, "off the shelf" form, since the rat does not reject the human tissue substance. Although, all the products claim to have significant osteoinductive potential, this study does demonstrate differences in their ability to induce an osteoinductive response capable of healing a spinal fusion. By at least 6 weeks and as early as 4 weeks, Osteofil and Grafton alone demonstrated posterolateral fusion manually, radiographically and histologically while Dynagraft, local human cortical bone, or autogenous bone did not.

	Local Bone			
	Dynagraft fusions/total	Grafton fusions/total	Osteofil fusions/total	Human fusions/total
Time of Sacrifice	0/1	0/1	0/1	-
2 weeks	0/5	2/5	4/5	-
4 weeks	0/7	7/7	7/8	0/4
6 weeks*	0/4	2/4	3/4	-
8 weeks	0/17	11/17	14/18	0/4
Totals				