PRO-STIM®
Injectable Inductive Graft

TECHNICAL MONOGRAPH
Proper surgical procedures and techniques are the responsibility of the medical professional. The following guidelines are furnished for information purposes only as techniques used by the design surgeons. Each surgeon must evaluate the appropriateness of the procedures based on his or her personal medical training and experience. Prior to use of the system, the surgeon should refer to the product package insert for complete warnings, precautions, indications, contraindications and adverse effects. Package inserts are also available by contacting Wright Medical Technology, Inc.

Please contact your local Wright representative for product availability.
PRO-STIM®
Injectable Inductive Bone Graft Substitute


» Accelerated Healing*
- Faster healing than autograft at 13 weeks in canine model

PRO-STIM® Injectable Inductive Graft is a resorbable, hardening, osteoinductive bone graft substitute. Built on the PRO-DENSE® material platform as a combination of calcium sulfate and calcium phosphate, PRO-STIM® graft adds demineralized bone matrix (DBM) for osteoinductive factors to speed the healing and remodeling process.

Autograft remains the “gold standard” for many surgeons because it includes all three desired features of a bone graft: scaffolding for osteoconduction, proteins for osteoinduction, and stem cells for osteogenesis. Additionally, it is autologous thus eliminating concerns of an immune or foreign body response. Donor morbidity and multiple complications at the site of autograft harvest, however, remain a challenge. While many materials have been shown to perform equivalent to autograft, two products to date- PRO-DENSE® graft and now PRO-STIM® graft- have demonstrated superiority to autograft in pre-clinical testing. In previous testing in a metaphyseal canine defect model, PRO-DENSE® graft demonstrated an ability to regenerate denser and stronger new bone faster than autograft at 13 weeks. Additionally, the new bone regenerate maintained increased density for a period before remodeling (by 26 weeks). As summarized in Section III PRO-STIM® graft demonstrated accelerated healing and earlier initiation of remodeling in treated sites compared to autograft in the same canine model.

*Canine proximal humerus model: Accelerated healing compared to autograft. Compared to autograft, PRO-STIM® Graft showed accelerated new bone formation at 13 and 26 weeks. This was evident by the slightly higher average stiffness, the greater amount of average new bone formation, and statistically significantly greater compressive strength shown for PRO-STIM® Graft treated defects. By comparing the 26-week clinical and contact radiographs and gross cross-sectional images and histological images, there appeared to be little to no apparent difference between defects filled with either PRO-STIM® Graft or autograft. Comparison of percentage of new bone, compressive strength, and modulus of elasticity showed no statistically significant difference between the materials at 26 weeks. It is unknown how results from the canine model compare with clinical results in humans. Data on file at Wright.
Self-Forming Porous Scaffold
Tri-phasic Resorption Reveals an Interconnected, Porous Scaffold

The physical progression of the resorption process is illustrated in FIGURE 1, which shows SEM images of cross sections through embedded PRO-STIM® graft after zero, two, four, eight, and twelve days of accelerated in vitro dissolution which has been estimated to occur about six times faster than in vivo. The darker region on the outer edge of the pellet represents the area in which calcium sulfate has largely dissolved, leaving a porous scaffold of brushite (bright white) and DBM particles (very dark spots).

FIGURE 1 | In vitro accelerated dissolution at 37°C in water (Image analysis via SEM) (Approximately six times faster than in vivo canine model).
In FIGURE 2, the SEM images of the pellets show that much of the size of the PRO-STIM® pellet is maintained at early timepoints. Even at 4 days, the calcium sulfate has started to dissolve revealing the porous calcium phosphate structure containing DBM particles. By 7 days, a large portion of the remaining pellet is the porous structure of calcium phosphate and DBM (large white particles). By contrast, pure calcium sulfate is completely resorbed by 10 days in this same experiment.

FIGURE 2 | *In vitro accelerated dissolution at 37°C in water (Image analysis via SEM)* (Approximately six times faster than in vivo canine model).

In FIGURE 2, the SEM images of the pellets show that much of the size of the PRO-STIM® pellet is maintained at early timepoints. Even at 4 days, the calcium sulfate has started to dissolve revealing the porous calcium phosphate structure containing DBM particles. By 7 days, a large portion of the remaining pellet is the porous structure of calcium phosphate and DBM (large white particles). By contrast, pure calcium sulfate is completely resorbed by 10 days in this same experiment.
Osteoinductive Potential

Osteoinductivity of PRO-STIM® Injectable Graft in an Athymic Nude Rat Model

Objective

The objective of this study was to evaluate the osteoinductivity of PRO-STIM® graft in an athymic rat muscle pouch model.

Materials and Methods

Incisions were made through the latissimus dorsi and gluteus superficialis muscles on each side of the midline of an athymic nude rat model. Two pouches were created on each side using blunt dissection. PRO-STIM® graft was mixed according to instructions. After aggressively mixing for 30 seconds, samples were manually rolled into small, elliptical balls. The graft specimens were gently implanted into the muscle pouches after they had hardened. Each rat received four implants. After implantation, the muscle incisions were closed. The implants were harvested at 28 days. Specimens were decalcified for ten days, embedded in paraffin, and stained with hematoxylin and eosin (H&E).

Results

The PRO-STIM® graft prepared with human DBM induced measurable new bone growth as shown in FIGURE 3.

Conclusion

PRO-STIM® graft was shown to be osteoinductive when implanted in an athymic nude rat muscle pouch model.

FIGURE 3 | Histology image of PRO-STIM® graft within athymic nude rat muscle pouch (H&E original mag 20X).
Healing with PRO-STIM® Injectable Graft Compared to Autograft in a Canine Critical Size Defect Model

Objective

The primary objective of the studies was to evaluate the in vivo performance, including at early timepoints, of PRO-STIM® graft in a canine proximal humerus critical size metaphyseal defect model. Defects were created, filled, harvested, and evaluated histologically at 2, 3, 4, 6, 8, 10, 13, and 26 weeks. A second objective was to compare healing in the defects treated with PRO-STIM® graft and autograft by compiling data from studies previously conducted in the same model, at the same institute, and by the same research team. Compressive strength of healed defects was also assessed at 13 and 26 weeks.

Materials and Methods

Cylindrical defects (13mm diameter x 50mm) were created bilaterally in the proximal humerus of adult male, hound-type dogs. Immediately prior to implantation, PRO-STIM® graft was prepared according to instructions provided with each kit. One defect per dog was filled with PRO-STIM® graft prepared with canine demineralized bone matrix (DBM) and the other defect was filled with autograft. Dogs were sacrificed at 2, 3, 4, 8 and 10 weeks (n=1 per timepoint); 6 weeks (n=3); and 13 and 26 weeks (n=5 per timepoint). Following sacrifice, the proximal humerus was harvested and the length of bone with the implant site was cut into sections. Contact radiographs were taken of sections of the defect. Specimens were processed and embedded in PMMA and slides were stained with basic fuschin and toluidine blue. The percentage of new bone was measured from histology images using a point counting method. Previous data from comparable defects treated with autograft were compared. From specimens harvested at 13 and 26 weeks, samples 8mm diameter x 20mm long were cored from the defect regions and loaded under compression until failure.

Results

Resorption of calcium sulfate, evident as darkening of the implant, starts at the outer rim of the implant and occurs within two weeks (FIGURE 4). Similar to the progression seen in vitro, the calcium phosphate and DBM initially remain within the resorbing area until surrounded and/or replaced by bone. Resorption and replacement with mature bone progresses quickly and the bulk of material, particularly the calcium sulfate and calcium phosphate, is gone by 8 weeks (FIGURE 4). In the
autograft implant sites, although a notable amount of new bone is present in the defect sites as early as 2 weeks, the new bone is markedly immature as indicated by thin, small spicules of young bone and remains largely immature past 8 weeks.

A. PRO-STIM® GRAFT

FIGURE 4 | (a) In contact radiographs of a cross-section of PRO-STIM® implant sites, resorption is evident by 2 weeks. By 6 weeks, the bulk of the material is gone and significant bone growth is evident. By 26 weeks, the entire defect site is filled with mature bone resembling adjacent native bone. (b) While new bone growth is evident as early as 2 weeks in the autograft implant sites, the thin bone appears very immature, without the density representative of more mature bone. This immature bone largely persists beyond 8 weeks.

B. AUTOGRAFT
Histological images of PRO-STIM® implant sites at each timepoint show distinct regions of implant resorption and healing (FIGURE 5) that progress inward over time.

Histology images of PRO-STIM® graft and autograft implant sites over time (FIGURE 6) show progression of material resorption and new bone growth consistent with views of the contact radiographs. As early as 2 weeks, new bone growth visibly maintains tight contact with the resorption front of the implant material. Consistent with contact radiographs, by 8 weeks, the bolus of implant is gone and the defect site has filled with new bone.

This rate of resorption and healing is consistent with the presence of remodeled mature cancellous bone in the defect site that is indistinguishable from surrounding bone at 26 weeks (FIGURE 6).
In the autograft sites, new bone is evident at 2 weeks although the bone is thin and spicule-like representing very immature bone which has little strength due to few, disorganized collagen fibers. This immature bone structure is maintained until 13 weeks at which time the bone has remodeled to more mature bone resembling surrounding bone.

A. PRO-STIM® GRAFT

![Images of PRO-STIM® graft at different time points](image)

B. AUTOGRAFT

![Images of autograft at different time points](image)

*FIGURE 6 | (a) Consistent with contact radiographs, at 2 weeks, resorption of PRO-STIM® graft with some evidence of new bone growth at the periphery of the implant is observed. Continued material resorption and notable new bone growth is apparent at 3 weeks and continues to progress at a fast rate with the bolus of the implant predominantly gone and replaced with mature bone at 8 weeks. [NOTE: The hole in 6-wk PRO-STIM® histology image is due to wash-out during post-explantation processing.](b) In the autograft sites, new bone observed at 2 weeks is very immature, indicated by thin, small spicules of young bone; remodeling into more mature bone does not occur until after 10 weeks. (Basic fuschin and toluidine blue, 10X)*
Graphical presentation of new bone growth over time (FIGURE 7) demonstrates acceleration of healing, increased volume in new bone formed, and remodeling to normal at 13 weeks with PRO-STIM® implants compared to autograft.

Mechanical test results indicated compressive strength comparable to that of normal bone in both PRO-STIM® graft and autograft-treated sites at both 13 and 26 weeks (FIGURE 8).

At 13 weeks, compressive strength of the PRO-STIM® specimens was comparable to normal bone and was significantly greater than that of autograft specimens (p=0.046) (FIGURE 8). The lower strength of the autograft sites may be due to decreased maturity of the new bone. By 26 weeks, the compressive strength of both the PRO-STIM® graft and autograft-treated sites was comparable to new bone.
Conclusion

PRO-STIM® graft has demonstrated accelerated healing, increased volume in new bone formed, and remodeling to normal bone at 13 weeks vs. autograft in the canine critical sized defect model.

PRO-DENSE® and PRO-STIM® grafts have been shown to be superior to autograft in the canine proximal humerus model at the 13 and 26 week time points.\textsuperscript{5,6}

PRO-STIM® graft demonstrates a resorption pattern that reveals the osteoinductive DBM over a defined period of time. The osteoinductivity was clearly demonstrated in the athymic nude rat muscle pouch model in which the composite graft was implanted and new bone formation was evident.

PRO-STIM® graft has demonstrated accelerated healing, increased volume in new bone formed, and remodeling to normal at 13 weeks vs. autograft in the canine critical sized defect model.
References


5. PRO-STIM® Graft Data on file at Wright Medical Technology.

Sizing and Ordering Information

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<td>PRO-STIM® CORE DECOMPRESSION KIT*</td>
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*Contains all necessary instruments for a standard core decompression technique.