Comparative Radiographic and Histological Evaluation of Collage™ and Vitoss™ in a Rabbit Radius Critical Defect Model

Introduction

Bone regeneration involves a sequence of complex, well-coordinated, biologic events, including but not limited to molecular signaling, which initiates intracellular and extracellular pathways, and osseous induction and conduction, in an attempt to regenerate bony tissue and repair defects.1-3

When the usual physiologic process of bone regeneration is either insufficient or impaired and requires additional therapy, clinicians must make an evidence-based and informed decision in order to select the appropriate therapeutic option to augment bone regeneration.

The generally accepted “gold standard” in bone grafting is the autologous bone graft (autograft). However, the autograft bone harvesting procedure may present complications, such as surgical site infection and chronic pain, and patients who have undergone previous autografting may have an inadequate supply of autologous bone.4-8

Synthetic bone graft substitutes are a therapeutic alternative to autografts and allografts and are constructed of biocompatible materials, which form scaffolds that promote bone regeneration.8,10 With the availability of multiple bone graft substitutes, clinicians bear a responsibility to evaluate the science and efficacy evidence. Whereas synthetic bone graft substitutes lack the osteoinductive properties of autografts and allografts, a product with osteoconductive properties and biocompatible materials, coupled with documented effectiveness, may present a viable treatment alternative without complications associated with autografts or allografts.8,10

Orthofix Collage™ Osteoconductive Scaffold putty consists of highly purified type-1 collagen, with inherent cell-binding capacity, high biocompatibility, and porous ß-tricalcium phosphate (TCP) granules in a 20:80 ratio (by weight).11 The microarchitectural combination of collagen and fast-resorbing ß-TCP granules results in an osteoconductive bone graft substitute that promotes vascularization and cell ingrowth.

The objective of this in vivo study was to compare histological and radiographic performance of an osteoconductive scaffold putty loaded with autologous bone marrow aspirate against similar and predicate devices of similar composition with respect to bone void filling.
**METHODS**

**Ethical Statement**
This study was approved by the Institutional Animal Care and Use Committee and was determined to be in compliance with applicable requirements of the Animal Welfare Act and pertinent state and local laws and regulations, and to be in adherence with the Public Health Service Policy and National Institutes of Health Guide, and to conform to USDA, NIH, AVMA, and AAALAC guidelines. All procedures involving animals were carried out in a humane manner under the direction of a trained individual.

**Study Design**
This was a preclinical, in vivo, surgical study conducted in 23 skeletally mature, female New Zealand White rabbits.

**Implants**
The following 4 products were used in this study: Collage β-TCP Putty, β-TCP Foam (Vitoss™)*, and two additional β-TCP & collagen based synthetics.*

**Experimental Procedures**

**Surgery:** Rabbits were randomized to the test or predicate treatment group, anesthetized by intramuscular anesthesia, and maintained on isoflurane with oxygen after intubation. After sterile cleansing and draping, a critical-sized defect measuring 1.5 cm in length was made bilaterally in each radius 2.25 cm proximal to the radiocarpal joint with an air-powered drill with a bone-cutting tip. Surgical sites were flushed, and critical-sized defects were confirmed.

**Bone marrow aspiration:** Bone marrow aspirates (BMA) were obtained from each medullary space via a 1-cm incision to the proximal right tibia. The total volume of the aspirate was confirmed to be at least 1.5 cc. BMAs were pooled after documentation of an adequate nucleated cell count.

**Implant:** Test articles and predicate devices were removed from packaging, cut to appropriate intraoperative dimensions, and saturated with pooled BMA. Caution was taken to ensure that loaded implants did not dry out before implantation. Implants were inserted into the bilateral critical-sized defects, ensuring adequate contact between implants and medial or proximal margins of bone before wound closure. No internal or external fixation was used.

**Radiographs/computed tomography scans**
Anterior and posterior radiographs were obtained immediately after the procedure by fluoroscopy. On the day of euthanasia (6 weeks or 12 weeks after implant), animals were lightly sedated to obtain forelimb AP radiographs and computed tomography (CT) images. CT scans were obtained with the animal in the prone position and the upper limbs positioned longitudinally. Radiographic healing was graded in a blinded fashion according to a grading scale (maximum possible score of 11) to assess the span of ossified callus (bridging), cortical remodeling (tissue regeneration), and visibility of ostectomy edges (remodeling of cortical bone).

**Histopathology**
Tissue specimens were processed for routine decalcified sectioning in paraffin, and longitudinal bone sections were obtained and stained by hematoxylin and eosin and trichrome. Blind quantitative assessment was conducted by semiquantitative scoring (maximum possible score of 23) and decoded group assessment to assess callus formation, proximal and distal bone union, graft resorption, and cortical remodeling.

*Orthofix Collage™ Osteoconductive Scaffold - Putty is substantially equivalent in function and intended use to Vitoss™ Scaffold Foam Bone Graft Matrix which has been cleared to market under Premarket Notification 510(k) K032288.
RESULTS
At 6 weeks, all defects showed evidence of early radiographic healing based on reviewer scoring (Fig. 3). Histopathology at 6 weeks showed evidence of repair of segmental defects in all implants, with evidence of callus spanning the breadth of the critical-sized defects (Fig. 1 and 2). By 12 weeks, repair of all defects had progressed substantially, regardless of the implant material used for repair, and histopathologic findings were equivalent for all implants, with structural repair as expected for bone void fillers in a 12-week surgical model. The bone void–filling properties of ß-TCP putty and similar formulations were comparable to those of ß-TCP foam based on semiquantitative and qualitative histopathologic assessments and blinded radiographic scoring.

DISCUSSION
The results of this study revealed that Orthofix Collage ß-TCP putty rapidly absorbed and retained autologous bone marrow aspirate and resisted compressive forces during implantation and throughout bone healing. When utilized with autologous bone marrow aspirate, the putty effectively healed critical bone defects at 6 and 12 weeks.

All defects showed a substantial increase in histologic scores from 6 to 12 weeks, and bone void–filling properties of the ß-TCP putty were equivalent to those of the other implants and the predicate device.

CLINICAL IMPLICATIONS
Orthofix Collage ß-TCP putty is a bone void filler which is effective when combined with autologous bone marrow aspirate, and its microarchitecture provides surgeons with a product that is easy to handle in the orthopedic surgical setting. Its clinical implications include the following:

• Lower risk of morbidity and complications associated with autograft and allograft
• Effective microarchitecture that enhances the osteoconductive properties of the graft by allowing fluid to be imbibed from soft tissue during healing

Animal Housing and Husbandry
Rabbits were monitored postoperatively without restraints and observed/treated for signs or symptoms of infection or pain. Animals were killed 6 and 12 weeks postoperatively.

Statistical Methods
Differences in radiographic and histologic healing scores between treatment groups and healing time points were evaluated by Wilcoxon nonparametric statistical analysis and a significance level of P = 0.05.
REFERENCES


Description

The Orthofix Collage™ Osteoconductive Scaffold – Putty (Orthofix Collage™ Putty) is a resorbable bone void filler made from a porous highly purified collagen matrix that has high purity beta-tricalcium phosphate (β-TCP) granules dispersed throughout. The implant is provided sterile, non-pyrogenic, for single use in double peel packages.

The Orthofix Collage™ Putty bone grafting construct is designed to facilitate the repair of bony defects. In the dry state, the matrix has a three-dimensional trabecular network of pores that resembles the pore structure of human cancellous bone. The Orthofix Collage™ Putty quickly imbibes fluids, making it easy to combine with bone marrow aspirate.

The Orthofix Collage™ Putty guides the regeneration of bone across the defect site into which the putty is implanted. New bone forms in apposition to the matrix surface when the graft is placed in direct contact with viable host bone. Ultimately the matrix is resorbed and remodeled into the bone.

Intended Use and Indications

Orthofix Collage™ Putty combined with bone marrow aspirate, is intended for use as a bone void filler to fill voids or gaps of the skeletal system in the extremities, spine, and pelvic not intrinsic to the stability of the bony structure. Orthofix Collage™ Putty is also indicated for use in the treatment of surgically treated osseous defects or osseous defects created from traumatic injury to the bone. Following placement in the bony void or gap (defect), Orthofix Collage™ Putty is resorbed and replaced with bone during the healing process.