

P-15™: A BIOMIMETIC BONE GRAFT SUBSTITUTE

Considerable research has gone into the development of acceptable bone graft substitute materials that can replace or augment autogenous bone grafts. These biocompatible materials have a varied form and function, but generally fall into the broad categories of being either osteoinductive or osteoconductive. Some materials are able to support functional loads while others, generally granular, or putty-like in nature, are intended for use in conjunction with mechanical support devices.

Recently a biomimetic bone matrix that simulates the cellular environment of hard tissue, identified as P-15™, was introduced to the orthopedic community [1]. P-15 is a synthetic fifteen amino acid residue, identical to the sequence (766)GTPGPQ-GIAGQRGVV(780) found in the $\alpha 1(I)$ chain of Type I collagen. Bhatnagar et al, have demonstrated that P-15, containing the potent cell-binding domain of collagen, can be adsorbed onto a calcium phosphate substrate, and will enhance cell attachment and extracellular matrix and factor production, resulting in the formation of bone and connective tissues.

P-15 bone graft substitute (ABM/P-15) is a combination of the mineral component of bone with a peptide replicating the cell-binding domain of Type-I collagen. The anorganic bone mineral (ABM) component provides the necessary calcium phosphate and the natural anatomical matrix needed for cellular invasion. The P-15 com-

ponent, a small synthetic peptide, modulates cell binding, migration, proliferation, and differentiation, as well as the synthesis and secretion of extracellular matrix elements and factors that facilitate the production of bone [2].

P-15: mechanism of action

The homeostasis of bone tissue is entirely dependent upon an exchange of ambient and biogenic mechanical stimuli. Biomechanical stimuli induce cellular processes that follow the lines of force (Wolff's Law). The flow of chemical and mechanical signals among cells, and between cells and their environment plays a crucial role in cell differentiation and morphogenesis [3].

Bone cells respond to mechanical cues by secreting growth factors and remodeling their surrounding matrix in an exquisitely orchestrated spatial and temporal program of matrix turnover and organization. Collagen is the

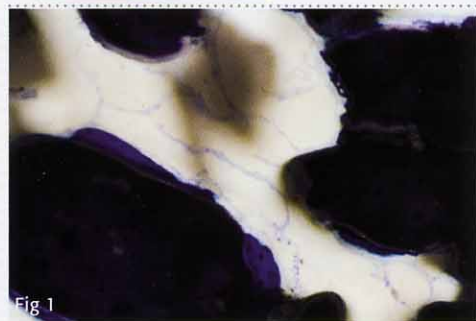


Fig 1

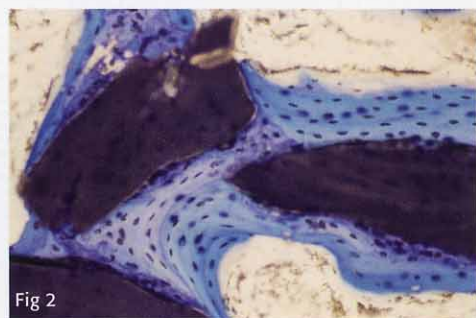


Fig 2

Histological sections (200x) of ABM (Figure 1) and ABM/P-15 (Figure 2) at 8 weeks, demonstrating increased bone formation of ABM/P-15 bone graft substitute.

most abundant protein in the body and it comprises over 95% of the organic component of bones. The immediate environment and scaffold of all anchored cells in bone is composed primarily of Type-I collagen. In tissues such as bone, Type-I collagen serves as the primary conduit for the bidirectional flux of regulatory signals. Cellular tractional forces contribute to the organization and orientation of the newly synthesized matrix, and these forces delineate the structural details for morphogenesis and remodeling.

Bone tissue (autograft) is a composite of inorganic calcium phosphates and Type-I collagen. Histologically, osteocytes are wrapped in an abundant and extensive network of collagen. The junction between cells and Type-I collagen serves to conduct mechanical stimuli. Thus Type-I collagen plays a mediating role, crucial to bone biology. By dissecting Type-I collagen and identifying P-15 as its site of contact with cells, scientists at the University of California – San Francisco were able to construct surrogates for collagen that mimic the behavior of collagen towards cells. ABM/P-15 is a biomimetic template and the efficacy of ABM/P-15 as a bone graft substitute arises from this “mechanotransduction” mechanism.

Studies [1] have shown that P-15, in combination with ABM, mimics the

behavior of autograft bone. ABM is entirely composed of calcium phosphate. Thus, the ABM/P-15 composite creates conditions for cells that mimic their habitat in tissues. In this biomimetic habitat, cells are able to pursue their physiological behavior including inter-cellular communications, which initiates the cascade of events leading to the formation of bone.

P-15 and bone repair—preclinical research

Ectopic bone formation

Concerns regarding the complication of ectopic bone formation have been raised for the commercially available growth factor products [4]. ABM/P-15 has demonstrated *in vivo* bone formation, with efficacy limited to bony sites, with no potential for ectopic bone formation. *In vitro* osteogenic HOS cells grown on ABM/P-15 demonstrated a significant increase in alkaline phosphatase (ALP) activity and expression of BMP-2 mRNA. In contrast, human smooth muscle cells grown on ABM/P-15 showed no increase in ALP or BMP-2 mRNA. Additionally, a standardized osteoinductive model using athymic rats compared the amount of bone formation in an ectopic (intramuscular) site with implants of ABM/P-15, ABM/P-15/CMC (carboxymethylcellulose gel carrier) and demineralized bone matrix (DBM) for up to 28 days.

ABM/P-15 and ABM/P-15/CMC demonstrated no ectopic bone formation while in contrast, DBM formed bone in all animals.

Cancellous bone defects

A defect repair study comparing ABM/P-15 in sodium hyaluronate carrier (ABM/P-15/Hy), Hy alone, ABM/Hy, or no graft was performed in drill hole defects in the proximal medial tibiae and distal femurs of rabbits. These treatments were evaluated histologically at 1, 2, 4, and 8 weeks for bone ingrowth. Empty or Hy filled defects had minimal amounts of new bone formation. At the sacrifice times of 2, 4, and 8 weeks, ABM/P-15/Hy had statistically significant greater new bone formation than the other treatments (Figs 1–2). New bone was in intimate contact with the ABM/P-15/HY particles with osteoid and adjacent areas beginning to mineralize at 2 weeks. There were many bone cells present within the new bone. In contrast the ABM/Hy graft showed substantially less bone and a smaller number of cells. The addition of P-15 significantly increased the rate of new bone formation.

Spine fusion

A three-level anterior cervical fusion study in goats investigated the use of ABM/P-15 in conjunction with cylindrical interbody spinal fusion cages. Twenty-four goats were fused using one of three methods: I) BAK cages coated with P-15 and filled with iliac crest autograft, II) BAK cages (no coating) filled with ABM/P-15, or III) empty, BAK cages coated with the P-15 peptide. Each group was evaluated at 3 months ($n=7$) and 6 months ($n=1$). Postmortem evaluations included radiographs, CT scans, ex vivo non-destructive flexion-extension biomechanical testing, histology, and microradiographs of the midsection of the cage. The microradiographs and histology indicated that bone formed earlier and that fusion rates were significantly higher in the ABM/P-15 filled cages (Group II) than the other

6-month micro-CT scans of iliac crest graft (Fig 3) and ABM/P-15 (Fig 4) demonstrating equivalent rates of fusion in the ovine lumbar spine model.

groups (fusion rates for Group I: 55%, Group II: 83%, Group III: 58%). In addition, the P-15 coating on the cages also improved the bone-metal interfaces as demonstrated in histology and by increased stiffness in flexion and extension during biomechanical testing. Due to the study design, direct comparison of ABM/P-15 and autograft, as a filler for interbody fusion cages could not be performed, since the cages with autograft were also coated with P-15, and the ABM/P-15 filled cages were not coated. However, the results from previous studies using the same model with autograft do suggest that ABM/P-15 may be at least as efficacious as autograft for filling fusion cages.

In a two-level sheep lumbar interbody fusion study, a direct comparison was made between ABM/P-15 and autograft harvested from the iliac crest. Six adult female sheep each received a two-level lumbar fusion using PEEK interbody spacers filled with either autogenous bone from the iliac crest or ABM/P-15. No additional spinal column stabilization was provided in order to better visualize bone formation. At 3 months, CT scans demonstrated that in both treatment groups, substantial new bone formed inside the PEEK interbody spacers, as well as, surrounding the PEEK interbody spacers with bone bridging the vertebral bodies. At the 6-month sacrifice time, all levels were judged fused based on CT scans, with further confirmation of fusion via micro-CT scans. (Figs 3–4) Total “fusion bone” area measurements made after 6 months indicated both graft treatments were similar in supporting spine fusions. In addition, micro-CT scans confirmed that the ABM/P-15 was undergoing active resorption, with little of the

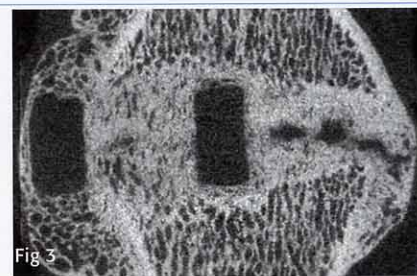


Fig 3

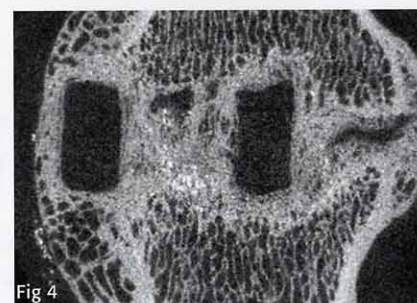


Fig 4

residual graft remaining after the 6-month evaluation period. This was the first time that ABM/P-15 demonstrated equivalence to autograft bone in a large animal spine fusion model.

Pilot clinical research

A pilot clinical study looking at the use of ABM/P-15 to treat long bone non-unions in 22 human patients was recently completed. All patients had failure of previous treatment with loss of internal fixation or lack of consolidation at least 6 months after initial treatment. Patients were divided into two separate series, I and II, and followed radiographically every 2 weeks until the first sign of callus formation and then monthly thereafter. Radiographs were evaluated by an independent surgeon for time elapsed for presence of bone bridging of major fragments and time to full consolidation.

Eight out of nine patients in Series I achieved full consolidation. The patient, who did not heal, experienced failure of the internal fixation hardware. At one-year postoperatively, the patient underwent a second surgical procedure. Additional ABM/P-15 was implanted and full union was achieved 3 months later.

Results from series II demonstrated full consolidation in 12 out of 13 patients. The average time to initial bone bridging was 1.5 months (range:

1–3.5 months) and the average time to full consolidation was 3.25 months (range: 2–5 months). Biopsies of fracture callus, taken from 2 patients at 13 months, showed active bone remodeling taking place with only a few particles of the ABM/P-15 present.

The results from these two clinical case series do demonstrate the excellent safety profile of the ABM/P-15 product over long implantation times and the potential for efficacy in a clinically challenging application. In the two series of patients in this study, 20 out of 22 patients (90%) had healing of their non-unions with one treatment of ABM/P-15. Autograft treatment of nonunions has been reported in the literature to have a union success rate of 74%, based on bone bridging in three radiographic views [5]. In addition, one of the two patients, who did not fully heal, was a result of a hardware failure. Though no statistical conclusions may be drawn, ABM/P-15 appears to offer a safe, and clinically useful alternative to autograft in the repair of long bone nonunions.

Summary

The 15-mer small synthetic peptide, P-15, adsorbed onto an anorganic calcium phosphate substrate, functions as an attachment site for anchorage dependent osteogenic cells. The attached cells respond to physical stimuli through a mechanotransduction process by producing and secreting growth factors and cytokines that facilitate the synthesis of bone and connective tissues. Pre-clinical studies in small and large animals have confirmed the beneficial effects of ABM/P-15 on bone formation and bone healing. A human pilot clinical trial for a trauma application demonstrated the safety and potential efficacy of ABM/P-15. This response lead to the initiation of a statistically designed, FDA approved, multi-centered, randomized, prospective, cervical spine discectomy and fusion clinical trial that is currently underway.

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