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-GIAQRGGV(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 component, 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 exquisitely 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
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), iby 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 significantly 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.

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 in bony sites, with no potential for ectopic bone formation [5]. In vitro osteogenic HOIs 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.

Spinous fusion
A three-level anterior cervical fusion study was performed using the use of ABM/P-15 in conjunction with cylindrical interbody spinal fusion cages. Twenty-four goats were fused using one of three methods: (1) BAK cages coated with P-15 and filled with iliac crest autograft, (2) BAK cages (no coating) filled with ABM/P-15, or (3) empty BAK cages coated with the P-15 peptide. Iliac graft was evaluated at 3 months (n = 7) and 6 months (n = 1). Posterior and anterior fusion rate evaluations included radiographs, CT scans, and a detailed evaluation of sagittal and coronal images. The addition of P-15 significantly increased the rate of new bone formation.

ABM/P-15 and ABM/P-15/CMC demonstrated equivalent rates of fusion in the osteoinductive model. This was the first time that ABM/P-15 demonstrated equivalent 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 bone graft and bone graft substitute. In total, 17 patients were included, 15 with long bone non-unions and 2 with intra-articular fractures. All patients were evaluated for clinical and radiographic outcomes at 6 months post-op.

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

Histological sections (200x) of ABM (Figure 1) and ABM/P-15 (Figure 2) at 6 weeks, demonstrating increased bone formation of ABM/P-15 bone graft substitute.
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. Preclinical 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.

References


THE AUTHOR
Vikas V Patel, MD, MA
Chief of Orthopedic Spine Surgery
University of Colorado, Denver, USA