●NOVEL CELL-BINDING PEPTIDE AS A BONE GRAFT SUBSTITUTE

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INTRODUCTION:

Type-I collagen is the most prevalent protein in the human body and is the primary organic component of human bone. Within the collagen molecule a sequence of 15 amino acids, (more specifically "GIAG") denoted the "cell binding domain of Type-I collagen" is responsible for cell-binding and subsequent organization of bone forming cells. When the peptide sequence is adsorbed onto a suitable matrix it acts as an attachment site for the α2β1 integrin on cell surfaces. This attachment initiates the cascade of intra-cellular signaling, cell differentiation, and subsequent osteogenesis. Thus, the combination of P-15 adsorbed onto a calcium based carrier creates a favorable micro environment for bone formation that is analogous to autograft bone. The ability of P-15 to passively enhance cell binding and subsequent cellular activation leading to bone formation represents a novel approach to facilitate bone growth. This is quite different from available bone growth factor formulations which utilize large amounts of rhBMP-2 or rhBMP-7 to stimulate cellular differentiation.

MATERIALS AND METHODS:

In order to produce a useable formulation of P-15, it is adsorbed onto anorganic bovine-derived hydroxyapatite (ABM). This yields a granular material which is then combined with a hydrogel, carboxymethylcellulose (CMC) to improve handling.

ABM particles have a diameter range of 250 μm to 420 μm. The ABM particles are slightly porous (28% total porosity) with a mean pore volume of 0.13 cc/g and small pore size (< 50 μm). The particles are produced using a high temperature (1100°C) process, yielding a completely deproteinated calcium phosphate matrix.

CMC is a derivative of cellulose and a well-known, biocompatible material that is used to maintain interparticular spacing. Combined with ABM/P-15 it yields a putty-like material with optimum handling characteristics.

ABM/P15 with or without CMC has been studied in multiple in-vivo models with subsequent clinical studies in dentistry and orthopaedic surgery.

RESULTS:

In Vitro Studies: The effect of P-15 on cell viability was studied using cultures of anchorage dependent human foreskin fibroblasts (HFF) and M3CTC-E1 preosteoblasts. At 2 and 4 days, cell survival was significantly greater on the ABM/P-15 material than on ABM for both cell types ($p < 0.05$). For the M3CTC-E1 cells, at 2 and 4 days of culture there were 5.1-fold and 15.2-fold greater cell viability with P-15, respectively, than on ABM alone. Concomitantly, apoptosis dropped 8-fold from day 2 to day 4 of culture with ABM/P-15 and increased slightly from day 2 to day 4 with ABM. When ABM/P-15 was compared to DBM with HFF cells in the presence of serum, the cell viability was approximately 7-fold greater on ABM/P-15 than on DBM, while at 2 days in the absence of serum there was no significant difference between the cell viability on ABM/P-15 and DBM.

Another *in vitro* study used human bone marrow stromal cells cultured on ABM or ABM/P-15 showed increased cell attachment by >50%, proliferation by 10-20%, ALP activity 2-fold in osteogenic media for up to 10 days, and collagen type I expression 2-fold at 10 days. BMP-2 expression was also significantly increased by the presence of P-15, reaching a 4-fold peak at 5 days as compared to ABM alone. By day 10, there was no significant difference in BMP-2 expression between the two groups, confirming the theory that BMP-2 is involved in the early phase of bone formation.

Rabbit Model: ABM was compared with ABM/P-15 in 20 rabbits with critical sized defects of 5mmX10 mm. At 2, 4, and 8 weeks histology and histomorphometry of the P-15 treated defects showed significantly increased bone formation compared with the control ($P>0.05$).

Goat Model: ABM/P-15 was utilized inside a titanium interbody cage in a three-level cervical fusion model involving twenty-four goats. At 6 months ABM/P-15 achieved an 83% fusion rate. In this model autograft historically obtains a fusion rate of between 40 to 50%.

Sheep Model: In this study, ABM/P-15 and autograft were compared in two-level uninstrumented lumbar fusions inside PEEK interbody rings in six adult sheep. In each animal, one level randomly received autograft and the other ABM/P-15. The graft material was held in place using a PEEK interbody ring. At 6 months both levels demonstrated equivalent rates of fusion and new bone formation as measured by micro-CT scans.

Clinical Dentistry: The particulate form of ABM/P-15 was evaluated for the treatment of periodontal defects in two prospective, controlled, multi-center clinical trials. ABM/P-15 was compared with surgical debridement, and DBM in study #1 and with ABM in study #2. At 6 months the P-15 treatment groups demonstrated a 72 - 73% defect fill compared to 51% with both control groups ($P<0.008$). Similar efficacy has been demonstrated in larger bone voids such as those that occur in sinus augmentation procedures in the oral cavity.

Clinical Orthopedics: A pilot clinical study evaluated ABM/P-15 and ABM/P-15/CMC in 22 patients with non-union or fracture malunion. Full consolidation was achieved in 90% of patients (20/22) as defined by radiographic bone bridging in at least three views. (Fig 1) The average time to consolidation was four months. Histological confirmation of bone formation was demonstrated as early as 4 months. (Fig 2) These results are comparable to those evaluating autograft and rhBMP-7.

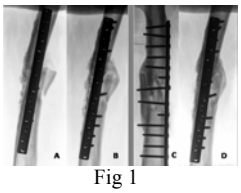

Fig 1

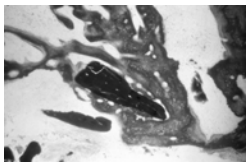
Fig 2

DISCUSSION:

ABM/P15 introduces a novel method of enhancing bone growth and fusion. It relies on an attachment factor that through the process of mechanotransduction, enhances cellular attachment, intra-cellular stimulation, and transformation; thus resulting in enhanced osteogenesis. It has now been tested in multiple animal and clinical models yielding enhanced bone formation and fusion when the material was introduced into the presence of potentially osteogenic cells.

In-vitro studies have demonstrated that P-15 increases attachment of osteoblast-like cells, which in turn produce BMP-2 and other bone growth factors. Expression of these bone growth factors promotes cell proliferation and differentiation and subsequent bone formation, as evidenced by the increase in alkaline phosphatase (ALP) activity when ABM/P-15 was compared to ABM alone and other bone graft substitute materials.

Based on the clinical results of ABM/P-15 in dental applications, two pre-clinical interbody fusion studies, and results from the pilot study in non-unions and malunions, P-15 bone graft substitutes have the potential to greatly improve the outcomes in orthopaedic bone grafting procedures and may be an effective, safe and economical alternative to- or adjunct with autogenous bone graft. Currently ABM/P-15 is the subject of an IDE multi-center pivotal trial for anterior cervical discectomy and fusion, comparing to autogenous bone.

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