SMALL PEPTIDE (P-15) BONE SUBSTITUTE EFFICACY IN A RABBIT CANCELLOUS BONE MODEL

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INTRODUCTION

The generation of a microenvironment that is a biomimetic of autogenous bone offers the potential for osseous regeneration. Recently, the use of small peptides in combination with a mineral matrix graft has shown that when placed in an osteogenic environment, it contributes to enhanced cell attachment, promotes osteoblastic differentiation, elicits deposition of matrix components, and integrates with the surrounding tissue.[1] It is speculated that this next generation bone substitute may be a valid alternative approaching the efficacy of the autograft. The small peptide, identified as P-15™, replicates the cell-binding domain of human Type-I collagen in the alpha 1(I) chain sequence ^{766}GTPGPQGIAGQRGVV^{780}. [2] Coating anorganic bone mineral (ABM) with P-15 (ABM/P-15) replicates the ligand role of native collagen in extracellular matrix and in wound repair.

To assess the efficacy of bone grafts in animal models, an osseous defect that will not heal spontaneously in the lifetime is usually advocated. However, for evaluation of enhanced bone formation, a delayed healing model is appropriate since early differences in bone formation can be identified. The purpose of the study is to evaluate the efficacy of the small peptide (P-15) by comparing the ABM matrix with and without the synthetic P-15 peptide in an inert hydrogel carrier in a cylindrical 4x10mm femur/tibia cancellous bone defect model.

MATERIALS AND METHODS

Twenty New Zealand male rabbits, weighing an average of 3.5±0.6kg, were used in the study. Rabbits randomly received either the ABM or the ABM/P-15 suspended in an inert hydrogel, and as controls, only the hydrogel and empty defects. Four defects (4mm diameter by 10mm depth) were created per rabbit in the distal femur and medial aspects of the proximal tibia.

At time periods of 1, 2, 4 and 8 weeks post surgery, five animals from each group were sacrificed. Femurs and tibiae were harvested *en bloc* and individual specimens fixed for histological analysis including: quality of new bone formation, interface characteristics of bone to implant materials and inflammatory response. Histological sections were quantified from total area of the circular defect obtained in each sample. Analysis of variance (ANOVA) using the Tukey-Kramer multiple comparison test evaluated the ABM/P-15 hydrogel treatment. The level of significance was set at $p < 0.05$.

RESULTS

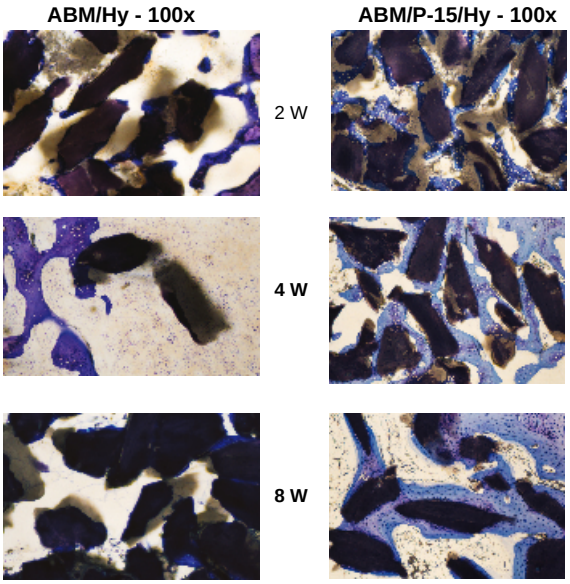
All the rabbits recovered with no negative response to the surgical procedure or graft material. All of the eighty defects were recovered.

Histology examinations: Sections receiving no graft material or hydrogel carrier showed minimal ingrowth with no inflammatory reaction associated with the hydrogel. No acute inflammatory infiltrate were visible for both the ABM and ABM/P-15 hydrogel grafts. At one week, bone formation was comparable but with increasing time. Histological sections at 2, 4, and 8 weeks showed greater bone formation with ABM/P-15 hydrogel. Figure 1 illustrates representative histological sections (100x) for each at 2, 4 and 8 weeks. Covariant analysis indicated that the treatment of differences were independent of the treatment location.

DISCUSSION

The significant results of this study may be directly attributed to P-15, which is an analogue of the cell-binding domain of Type-I collagen. The efficacy of the P-15 small peptide is the result of an autocrine response in contrast to the addition of exogenous growth factors, such as bone morphogenic proteins. P-15 is believed to enhance bone formation by facilitating cellular attachment with subsequent increase in cell binding, proliferation and differentiation of cells increasing TGFß-1, BMP-2, and BMP-7 levels that could positively influence all processes of new bone formation. The P-15 provides a biomaterial that has the ability to interact with cells by a mechanism (mechanotransduction) that simulates the interaction with cells with their extracellular matrix[3], e.g. , mimicking the role of collagen.

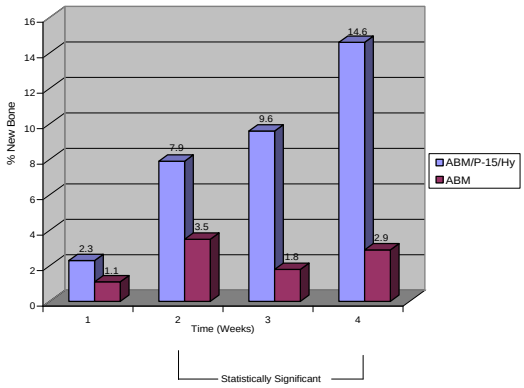
Figure 1



HISTOMORPHOMETRY

Bone formation of the ABM/P-15 hydrogel group was significantly greater than the hydrogel in all four defect positions and at sacrifice times of two weeks or more (Figure 2).

Figure 2



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