

Bone Tissue Engineering with Multiple-Factor Delivery Platform

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Introduction

Tissue development and regeneration is regulated by an interplay among various tissue inductive growth factors, formation of an appropriate vascular bed to support the metabolic needs of the forming tissue mass, and a cell population capable of responding to the chemical cues and creating the new tissue. There has been considerable interest in understanding this signaling interplay in bone, due to its limited ability to heal upon serious fracture or trauma and the limitations in common treatments for bone morbidity or replacement. Bone morphogenetic proteins (BMPs) are responsible for initiating cartilage and bone progenitor cell differentiation [1], and sequencing new bone formation via endochondral ossification [2]. Vascular endothelial growth factor (VEGF), a potent angiogenic molecule, appears to be particularly important in bone formation [3, 4]. Ultimately, these factors must act on a population of cells capable of responding to local factors and forming bone tissue. Multipotent stem cells originating from the bone marrow stroma, or bone marrow stromal cells (BMSCs), are a particularly attractive source for osteogenic precursors for bone tissue engineering [5] as they can be easily harvested and expanded *in vitro*, and induced to differentiate into bone forming cells. We report a material system that allows combined delivery of inductive molecules and cells without the need for viral vector-mediated *ex vivo* or *in vivo* genetic engineering. This scaffold delivery system is capable of combined delivery of condensed plasmid DNA encoding for BMP-4, VEGF, and human bone marrow stromal cells from biodegradable polymer to promote bone formation.

Experimental

Preparation of PLGA Scaffolds

PEI DNA condensates were prepared by combining 200 µg of plasmid in 4 ml Hepes buffer (5 mM, pH 7.4) with 4 ml of PEI (10mM). Freeze dried PEI DNA condensates were combined with milled sucrose and 85:15 PLGA copolymer particles to fabricate scaffolds as previously described [6]. For sponges containing vascular endothelial growth factor (VEGF), 250µl of 1% alginate solution in ddH₂O and 3µg of VEGF were vortexed and lyophilized overnight before mixing with PLGA, sucrose and condensed DNA prior to the gas foaming process.

In vivo Bone Formation and Analysis

The ability of the combined delivery system to enhance bone formation *in vivo* was assessed by the implantation of PLGA sponges containing combinations of condensed DNA, VEGF, and human bone marrow stromal cells into the subcutaneous tissue of SCID mice. The plasmid DNA used was pcDNA3-BMP-4. Eight experimental conditions were used: (1) blank scaffolds, (2) scaffolds delivering VEGF only, (3) scaffolds delivering hBMSCs only, (4) scaffolds delivering BMP-4 only, (5) scaffolds

delivering both hBMSCs and VEGF, (6) scaffolds delivering both BMP-4 and VEGF, (7) scaffolds delivering both BMP-4 and hBMSCs, (8) scaffolds delivering BMP-4, VEGF and hBMSCs. Elastic moduli of the retrieved specimens (N=6) were measured using compression tests performed at a constant displacement rate of 1mm/min. After the mechanical testing, each implant was fixed and then moved to 70% ethanol before performing histological analysis.

Results

Examination of histological sections from von Kossa staining confirmed that no mineralized tissue was observed in blank scaffolds and scaffolds delivering VEGF at 15 weeks (Figure 1, A and B). Scaffolds delivering BMP-4 only and scaffolds delivering hBMSCs only demonstrated small nodules of mineralization, sporadically scattered throughout the scaffold area (Figure 1, C and D). Scaffolds delivering both VEGF and hBMSCs demonstrated small regions of mineralization (Figure 1E). Scaffolds delivering both BMP-4 and hBMSCs and scaffolds delivering both BMP-4 and VEGF demonstrated an increased number of mineralized regions relative to the previous conditions (Figure 1, F and G). Combining BMP-4 and VEGF delivery and transplantation of hBMSCs demonstrated extensive mineralized tissue formation (Figure 1H).

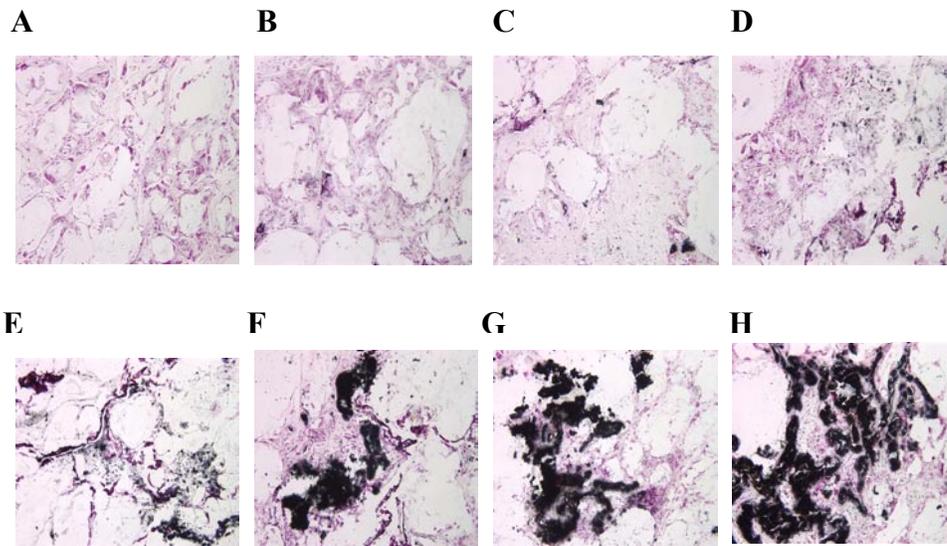


Figure 1. Photomicrographs of fifteen-week sections from each condition stained with von Kossa stain. All photomicrographs were taken at 200 \times .

To determine if the combined delivery of all three components also increased the quality of newly formed bone tissue, the elastic moduli of the implants were determined at fifteen weeks. Constant strain rate compression tests revealed significantly higher elastic moduli in the scaffolds delivering condensed DNA, VEGF and hBMSCs compared to other conditions (Figure 2).

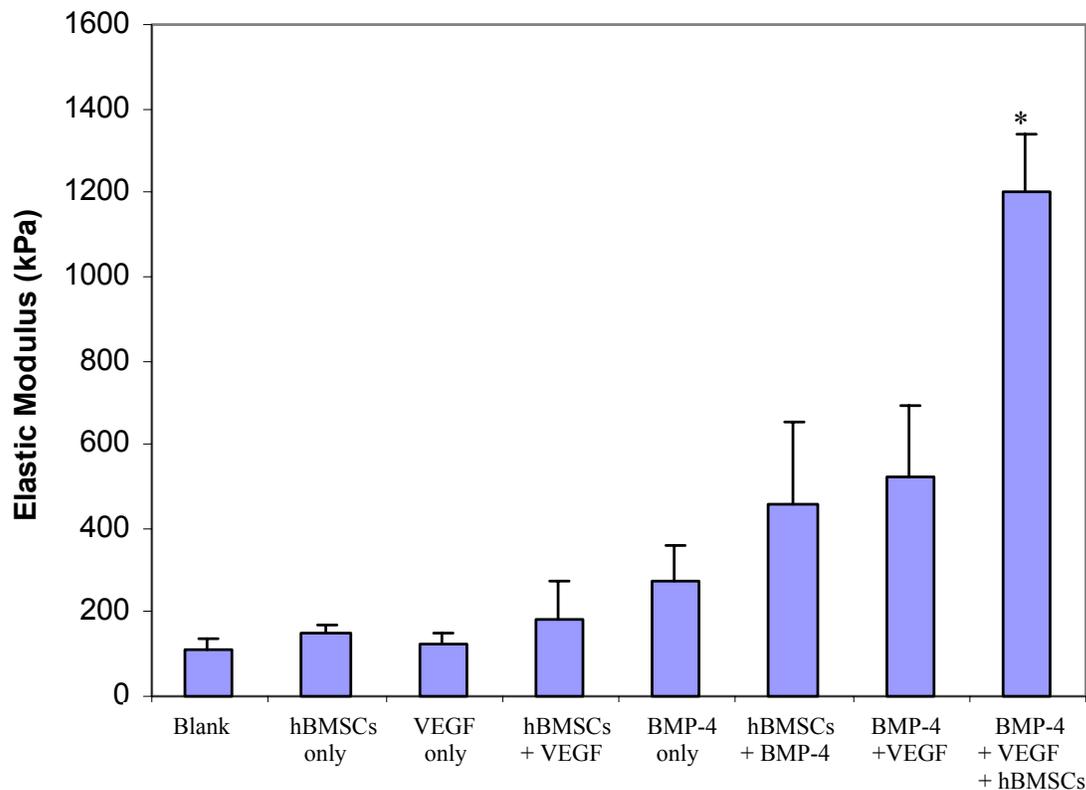


Figure 2. Elastic moduli of engineered bone for each condition at fifteen weeks.

Discussion

The effects of the combined local and sustained presentation of an osteogenic and angiogenic growth factor with a cell population competent to form bone were examined in this study. Any combination of the factors displayed increased bone formation, when compared to individual factors alone, demonstrating their interactive roles in bone development. Plasmid DNA potentially can be taken up by both hBMSCs and the infiltrating surrounding host cells (e.g. fibroblast). The BMP-4 secreted by both cell sources likely acted in both autocrine and paracrine manners by directly differentiating the hBMSCs into osteoblasts, and initiating the differentiation of osteoprogenitor cells from the host tissue into bone forming cells. The delivery of VEGF greatly accelerated and enhanced bone regeneration resulting from a combination of osteogenic growth factor and hBMSCs, emphasizing the critical role of this molecule in bone regeneration [7]. The greater moduli observed in this condition is likely related to the increased bone volume in these tissues, thicker bony trabeculae, and increased interconnectivity of the bone microarchitecture.

This study demonstrates the importance of combining multiple factors in bone regeneration. This system may be broadly useful in engineering a variety of other tissue types, as regeneration of all tissues is dependent on the interplay of various growth factors and cell types. The ability of this system to deliver proteins, (e.g., VEGF in current study), or plasmid DNA creates significant flexibility for controlling the dose and

duration of the delivered factors. This combined scaffold delivery system may find therapeutic applications and serve as a model system to study the interplay between factors involved in bone formation and engineering of other complex tissues.

References

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