Modified RNA Activated Matrices Enhance Bone Regeneration
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Purpose: Here we propose a novel delivery system with the potential to overcome many of the barriers associated with protein as well as DNA based therapeutics for bone regeneration. Employing inexpensive biomaterials to embed and release chemically modified ribonucleic acid (cmRNA) in a controlled manner addresses the high cost and safety concerns that exist with recombinant protein and viral based approaches. By eliminating the need for nuclear trafficking[1] (the ultimate barrier for successful transfection in non-dividing cells), cmRNA delivery could potentially address the lower transfection efficiencies associated with non-viral gene delivery systems and, since this strategy employs non-viral vectors[2], it alleviates the immunogenic concern that exists with viral vectors.

Methods: The complexes of polyethylenimine (PEI)-cmRNA and PEI-pDNA (encoding BMP-2) were fabricated at an amine (N) to phosphate (P) ratio of 10 and characterized for transfection efficacy in vitro using human bone marrow stromal cells (BMSCs). They were also characterized for size and surface charge using a Zetasizer Nano ZS. The in vitro cytotoxicity and transfection efficacy of the synthesized complexes was evaluated in bone marrow stem cells (BMSCs) using an MTS assay and by quantifying the amount of secreted BMP-2 in cell culture supernatants using ELISA. The osteogenic potential of BMSCs treated with these complexes was determined by expression of bone-specific genes, osteocalcin and alkaline phosphatase as well as through the detection of bone matrix deposition. The in vivo functional potency of collagen scaffolds loaded with either PEI-cmRNA complexes or PEI-pDNA complexes was evaluated in critical-sized calvarial defects in Fisher 344 rats.

Results: The PEI-cmRNA complexes were approximately 153 nm in size with a narrow size distribution. At the concentrations used for transfection, the complexes displayed low cytotoxicity and effective transfection as assessed by the MTS assay and ELISA, respectively. Alizarin red and Von Kossa staining demonstrated enhanced osteogenic differentiation as evidenced by increased bone matrix production by BMSCs transfected with PEI-cmRNA complexes. Using a calvarial bone defect (CBD) model in rats it was shown that PEI-cmRNA (encoding BMP-2)-activated matrices promoted significantly enhanced bone regeneration compared to PEI-plasmid DNA (BMP-2)-activated matrices (figure 1).

Conclusions: There exists a dire need for improved therapeutics to achieve predictable bone regeneration. Here, we demonstrate the significant bone regeneration capacity of PEI-cmRNA (encoding BMP-2) activated matrices in rat calvarial defects (at 4 weeks), compared to PEI-plasmid DNA (BMP-2)-activated matrices. Our study suggests that non-viral cmRNA activated matrix encoding osteogenic molecules can provide a powerful strategy for bone regeneration with significant clinical translational potential.

References: