MicroRNA 29a Inhibitor Loaded Gelatin Nanofibers for Localized Gene Therapy

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Statement of Purpose: Nanofiber scaffolds are attractive for bone tissue engineering, as they closely mimic the morphology of collagen fibrils in the natural extracellular matrix (ECM). MicroRNAs (miRNAs, miRs) are important regulators of bone maintenance and formation, and have emerged as powerful new therapeutic molecules. However, efficient tools to deliver miRNA mimics or antisense oligonucleotide inhibitors (antagomirs) to specific target tissues are limited. The miR-29 family is well studied in bone. miR-29 inhibits the synthesis of ECM molecules, such as fibrillar collagens, as well as the non-collagen matrix protein, osteonectin. Osteonectin regulates collagen fibril assembly, and is critical for normal bone remodeling. Inhibiting miR-29 activity increases ECM synthesis. The objective of this study is to develop a localized gene therapy for bone regeneration by combining nanostructured scaffolds with miR-29a inhibitors, to enhance the production of ECM. We evaluated the ability of these scaffolds to increase ECM production by quantifying osteonectin in vitro.

Methods: Gelatin was dissolved in trifluoroethanol to obtain a 7.5% (w/v) solution. Scramble (control) or miR-29a inhibitor with TKO transfection reagent at a ratio 1:1 was added to the gelatin solution, to yield concentrations of approximately 50nM/scaffold. Electrospinning was performed at a voltage of 10.5 kV, a distance of 10 cm and a flow rate of 0.8 ml/hr followed by crosslinking using glutaraldehyde. The encapsulation of fluorescently labeled scramble miRs in the gelatin nanofibers was visualized by fluorescent microscopy. Release kinetics of miR-29a inhibitor was determined by incubating scaffolds in PBS for up to 72hrs. Released miRNA inhibitor was quantified by NanoDrop spectrometry. Toxicity of miR-29a inhibitor loaded nanofibers was determined by MTS Assay. The ability of miR-29a inhibitor loaded fibers to regulate gene expression was assayed using the pre-osteoblastic cell line MC3T3-E1.

Results & Discussion: TRITC-labeled miRNA inhibitor encapsulated in electrospun gelatin nanofibers were uniform and bead-free (Figure 1). Sustained miR-29a inhibitor release from the gelatin nanofibers was observed over a period of 72hrs (Figure 2). miR-29a negatively regulates osteonectin by binding to its mRNA, causing instability and disrupting translation. Thus, introducing a miR-29a inhibitor will enhance osteonectin expression. Western blot analysis showed that MC3T3-E1 cells cultured for 24 hrs on miR-29a loaded gelatin fibers had significantly increased expression of osteonectin (Figure 3B, C). MTS assay demonstrated that the fibers were non-toxic, and had a similar number of cells (Figure 3A). Overall, these data indicate that miR-29a inhibitor remained active after encapsulation, entered the cells and was able to regulate gene expression in a manner similar to traditional transfection methods.

Conclusions: The study demonstrated the feasibility of producing miR-29a inhibitor loaded nanofibers as osteogenic scaffolds. Gelatin nanofibers locally delivered bioactive miR-29a inhibitor in a sustained manner, inducing the expression of the critical ECM component, osteonectin. Applications for this novel technology include the ability to deliver transient RNA-based gene therapy, without potential for cell transformation. Further, this approach is flexible, with the potential to deliver any miRNA inhibitor or mimic. The unique bioactivity of miRNA-based therapeutics, combined with ECM mimicking nanostructured scaffolds serves as a novel platform for localized gene therapy for bone regeneration.

References