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)[1]. microRNAs (miRNAs, miRs) are important regulators of bone maintenance and formation, and have emerged as powerful new therapeutic molecules[2]. 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[2]. 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[2]. 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[2]. Thus, introducing a miR-29a inhibitor will enhance osteonectin expression[2]. 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 3 B, 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.

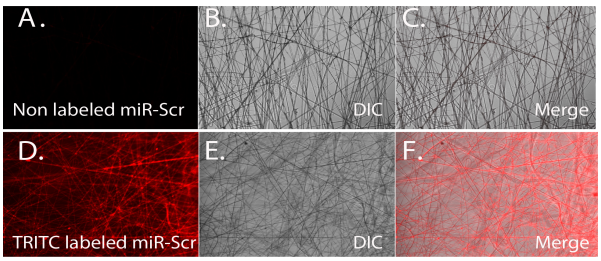

Figure 1. Fluorescent images of TRITC labeled and non-labeled Scramble miRNAs in gelatin nanofibers. Similar morphology was noted.

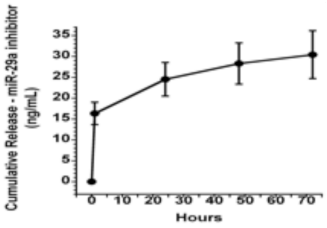

Figure 2. Release kinetics for miR-29a inhibitor

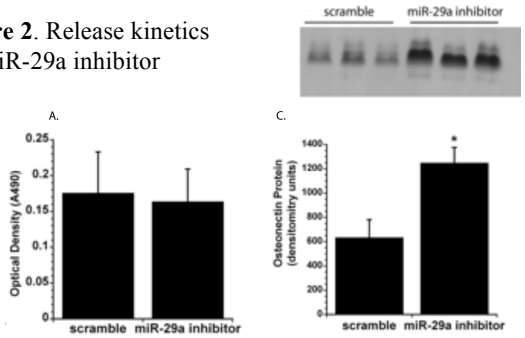

Figure 3. A. MTS assay at 24hrs shows similar viability in both groups. **B**. Western blot analysis of osteonectin in conditioned medium after 24hrs. **C.** Quantified Western data. *=p<0.01

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

1. Singh H, James E, Kan H, Nair LS. J Biomat Tissue Eng. 2012;2:228-235
2. Kapinas K, Kessler CB, Delany AM. J Cell Biochem 2009;108;216-24