Research Week 2020

Role of Cellular Microenvironment in Intracellular mRNA Delivery for Applications in Tissue Engineering

Avathamsa Athirasala, B.Tech., M.S., Siddharth Patel, Paula dos Passos Menezes, Gaurav Sahay, Luiz Eduardo Bertassoni

OHSU

Keywords

Tissue engineering, Mechanotransduction, mRNA

Abstract

Introduction

The use of synthetic mRNA-based gene modification offers several advantages over traditional DNA based gene therapy including faster translational kinetics, transient expression and mitigation of risks associated with insertational mutagenesis and genomic integration. Accordingly, mRNA technology holds particular promise for applications in tissue engineering where spatiotemporal control of gene expression is crucial. Recent advances in non-viral delivery technologies have significantly increased the efficiency of intracellular delivery of synthetic mRNA by modulating mTOR signaling, which has been identified as a crucial player in mRNA translation. However, mTOR signaling is regulated by matrix stiffness through mechanotransductive pathways. Therefore, the mechanical properties of the engineered tissue must be tailored for optimal translation and expression of mRNA.

Methods

Human Mesenchymal Stem Cells (hMSCs) seeded on plastic culture dishes were transfected with 100 ng/ml Green Fluorescent Protein (GFP) encoding mRNA packaged into lipid nanoparticles (LNP). 4 hours post transfection, the cells were trypsinized and encapsulated in GelMA (7% (w/v)) hydrogels constructs at a density of 2 x 106 cells/ml in order to study mRNA expression in physiologically relevant 3D models. The mechanical properties of the hydrogel constructs were modulated by photopolymerization for 25, 50, 75 and 100s (blue light, 20 mW/cm2). GFP expression was measured at 6, 24, 30 and 48 hours post transfection by fluorescence microscopy. mTORC1 activation was measured by intensity of fluorescence signal from immunostaining and imaging with Confocal Microscopy. Mechanical signaling inhibition studies used a ROCK inhibitor (Y27632, 30 mM).

Results

We observed a stiffness dependent response expression where intensity and efficiency of expression of GFP reduced with stiffness of the hydrogels. Lower expression of the mRNA in stiffer hydrogels correlated with reduced activation of mTORC1. When mechanical signaling was inhibited, both mRNA expression as well as mTORC1 activation were significantly reduced irrespective of stiffness of the hydrogel. The results show that the cellular microenvironment plays a significant role in the mechanisms of intracellular delivery and translation of synthetic mRNA with significant implications for both design of in vitro models as well as for translational applications.