Proceedings of the World Congress on New Technologies (NewTech 2015)
Barcelona, Spain, July 15-17, 2015
Paper No. 466

Light-Induced Gene Silencing for Applications in Regenerative Medicine

Chad Greco, Thomas H. Epps, III, Millicent O. Sullivan

Extended Abstract

The dynamic modulation of gene expression to illuminate and manipulate cell growth and signaling is an essential aspect in tissue engineering technologies, yet presents a particular challenge in complex tissues that must control phenotypes of multiple types of cells in a region-specific fashion. Specifically, methods to spatiotemporally control gene expression by cells growing within complex tissue scaffolds would provide an essential complement to current biomaterial strategies employed to manipulate cell behavior *via* extracellular signals. Multiple strategies have been explored for artificial regulation of gene expression in eukaryotic cells, including inducible promoter systems and optogenetic regulation strategies; these systems offer tremendous potential, yet the approaches used do not address the significant difficulties in delivering regulatory components to cells. As an alternative, approaches employing stimuli-responsive nanomaterials have shown promise in generating cellular responses with some degree of spatiotemporal control; however, off-target effects and limitations in efficacy continue to plague these systems. To address these challenges, we have developed novel and tailorable mPEG-*b*-poly(5-(3-(amino)propoxy)-2-nitrobenzyl methacrylate) [mPEG-*b*-P(APNBMA)]-based block copolymers (BCP)s with proven biocompatibility, stable and tunable nucleic acid binding, and light-triggered side chain cleavage leading to rapid and on-demand nucleic acid release. Herein, we report the ability to exploit these materials for spatiotemporally controlled deployment of siRNA cargoes, resulting in the ability to locally "dial-in" precise patterns of gene silencing in NIH/3T3 and adventitial fibroblast cultures. Gene silencing efficiency could be tuned over a range of approximately 0 – 85%, on the basis of varied light irradiation and varied polyplex composition, and spatially controlled gene expression exhibited cell-to-cell accuracy and no observable off-target effects. Using fluorescence correlation spectroscopy and fluorescence resonance energy transfer, we demonstrated that the variations in silencing efficiency were directly controlled by light-induced changes in polyplex structure leading to siRNA release within the cytoplasm, and we show that the polyplexes remain entirely dormant in the absence of illumination. Through development and application of mass action kinetic modeling, we show that the maximal silencing efficiency is defined by a pseudo-steady state balancing the rates of mRNA production and siRNA/RISC-mediated mRNA cleavage. This work establishes the framework for addressing a key challenge in regenerative medicine while also exploring the fundamental mechanisms of nanomaterial intracellular stability and delivery barriers.