Localized and Sustained Delivery of siRNA from Biopolymer Hydrogels

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Statement of Purpose: RNA interference is a powerful gene silencing mechanism which has the potential to revolutionize disease treatment and aid in the functional repair of damaged tissue by decreasing the expression of specific proteins. However, effective delivery of short interfering RNA (siRNA) to target cells remains a significant challenge to realizing its full therapeutic potential as siRNA is highly prone to degradation by ubiquitous RNAses, its targeting and retention at a specific location is problematic, and the silencing effect may last only a few days in rapidly dividing cells. Here, we present a new method for delivery whereby three-dimensional macroscopic biopolymer scaffolds retain siRNA locally and release it in a sustained manner to prolong the effect directly at the site of interest.

Methods: siRNA against GFP was mixed into 2% alginate or 3 mg/ml collagen, with or without HEK293 cells stably transfected with destabilized GFP. The hydrogels were crosslinked in a transwell membrane, either by addition of calcium (alginate), exposure to UV light (photo alginate), or by incubation at 37°C (collagen). siRNA release from the hydrogels was measured using RiboGreen®. Bioactivty of released siRNA was confirmed by flow cytometry of transfected cells. Additionally, the bioactivity of the siRNA within the hydrogel was examined by co-encapsulating the HEK293 cells with the siRNA and examining the GFP expression over time by confocal microscopy.

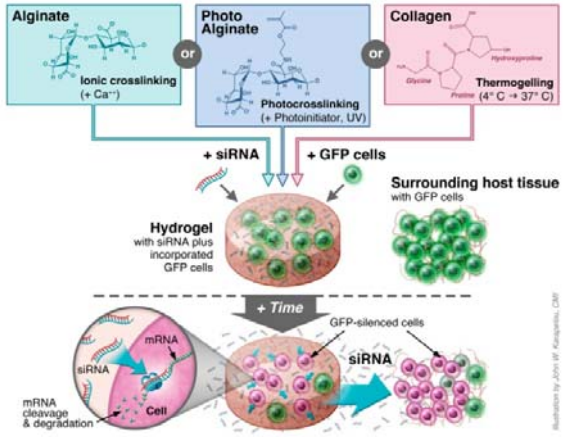

Figure 1. *Schematic of hydrogel formation for delivery of siRNA and subsequent inhibition of gene expression in incorporated and neighboring cells.*

Results: The siRNA was shown to silence GFP expression in HEK293 cells plated in monolayer (data not shown). The release of siRNA from hydrogels was examined. The siRNA diffused from the calcium-crosslinked alginate gels for approximately 5 days, and the amount released was decreased by the addition of positively charged polycations such as chitosan or PEI. (Fig. 2a). Almost all of the siRNA was released from the photo alginate during the first week; in contrast, the collagen hydrogels retained much of the siRNA (Fig. 2b). The calcium-crosslinked alginate, photo alginate, and collagen all released bioactive siRNA capable of silencing GFP expression to at least 6 days (Fig. 2c,d). Cells co-incorporated with the siRNA into the hydrogels show GFP silencing at 3 days (Fig 3). This effect was not maintained at 6 days for the photo alginate, but the collagen still exhibited substantial knockdown by day 6. The effect was more sustained for the cells cultured with the siRNA in the hydrogels compared to the siRNA delivered exogenously one time in the media.

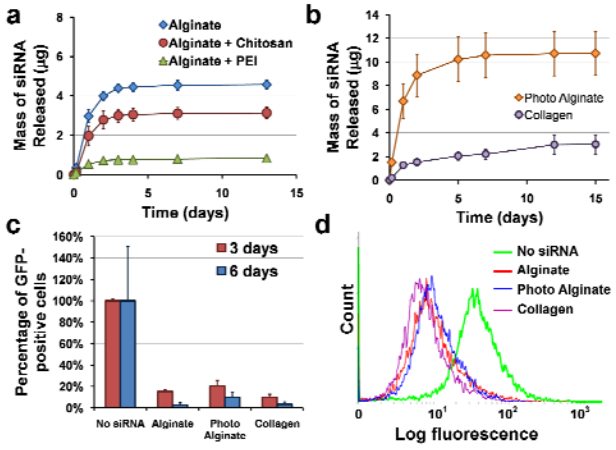

Figure 2. *Release and bioactivity of siRNA from hydrogels. (a,b) Cumulative release of siRNA from alginate and collagen hydrogels. (c) Percentage of GFP-positive cells after 3 and 6 days, normalized to no-treatment controls. (d) Flow cytometry histograms of samples show GFP silencing at day 3.*

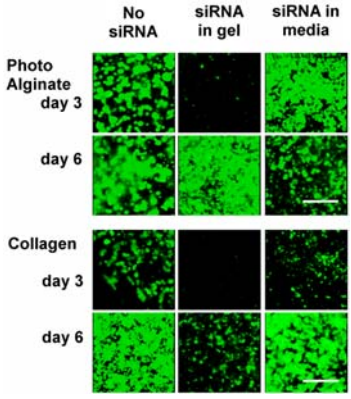

Figure 3. *Confocal fluorescent micrographs of cells cultured in three-dimensional hydrogels exposed to no siRNA, siRNA only in the hydrogels, or siRNA present in the media for only the first 24 hours of the experiment.*

Conclusions: Localized and sustained delivery of bioactive siRNA can be achieved from alginate and collagen hydrogels. Our laboratory is currently investigating the use of controlled siRNA release for tissue regeneration and disease therapeutics.

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