Photocleavable hydrogels for light-triggered siRNA release

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Statement of Purpose: RNA interference (RNAi), which harnesses, for example, short interfering RNA (siRNA) to inhibit gene expression post-transcriptionally by targeted destruction of specific mRNA molecules, has been gaining great interest for applications in cancer treatment and regenerative medicine [1,2]. siRNA has been delivered systemically to cells in vivo using nano- and microparticles; however, these particles disperse rapidly and are hard to target to a specific cell population. In contrast, macroscopic hydrogels permit localized and sustained delivery of bioactive factors. Controlled, localized and/or prolonged delivery of siRNA from hydrogels has been achieved through regulation of release via siRNA diffusion, hydrogel degradation and/or affinity interactions [1,3-5]. Here, we present an engineered photolabile hydrogel system for on demand delivery of siRNA upon exposure to ultra violet (UV) light, which photolytically degrades the crosslinked network to release bioactive agents more rapidly than without UV exposure.

Methods: Poly(ethylene glycol)-di(photolabile acrylate) (PEG-DPA) was synthesized as previously reported [6]. Accell siRNA against green fluorescent protein (siGFP) (13.3 µg) (Thermo Scientific Dharmacon, Lafayette, CO) was mixed with amino ethylene methacrylate (AEMA, 160 µg) prior to combining with PEG-DPA solutions. Ammonium persulfate and tetramethyl-ethylenediamine as redox initiator and catalyst, respectively, were then added to the mixture for crosslinking, resulting in hydrogels (50 µl, 15 wt%). siRNA release was performed in DMEM in the absence and presence of UV light (10 mW/cm^2, 20 min) at each time point. Released siRNA was quantified using a RiboGreen assay. To examine the bioactivity of released siRNA, destabilized GFP (deGFP) expressing HeLa cells in monolayer were cultured with the release samples (0.5 µg siRNA/well) for 48h before the cells were harvested for flow cytometry to quantify the degree of GFP silencing. siRNA against luciferase (siLuc) released from hydrogels was used as non-targeting control siRNA. The GFP expression of all samples was normalized to controls. Statistical analysis was performed using ANOVA with Tukey–Kramer Multiple Comparisons Test with $p<0.05$ considered statistically significant.

Results: Upon gelation, siRNA was retained within the hydrogel network via electrostatic interactions with primary amine groups of AEMA. To examine the capacity of the hydrogel system to release siRNA in response to UV light, hydrogels containing siRNA were exposed to UV light and the results were compared to those without UV application. Hydrogels without UV treatment ("siGFP, No UV") gradually released siRNA over 40 days (Fig. 1A) at which point the gels were completely degraded via the hydrolysis of ester linkages within the hydrogel network. In contrast, release of siRNA was accelerated upon the application of UV light to the hydrogels ("siLuc, UV" and "siGFP, UV"), as a result of their photodegradation. The hydrogels with UV application degraded completely within 28 days compared to 40 days without UV application. At the same hydrogel concentration and UV conditions, the release profile of non-targeting control siLuc was similar to that of siGFP. siRNA released in response to light was controlled by regulating hydrogel concentration, UV intensity and UV exposure time (data not shown). The bioactivity of the released siRNA was then examined by presenting the released siRNA to deGFP expressing HeLa cells. Cells without any treatment (Control), and those treated with release from hydrogels without siRNA ("Gel only, UV") or containing control siLuc ("Gel, siLuc, UV"), expressed ~100% GFP (Fig. 1B). However, when applying the same siRNA concentration, siGFP released from no UV ("Gel, siGFP, No UV") and UV ("Gel, siGFP, UV") treated hydrogels could knockdown GFP expression by 48.70 and 42.45%, respectively. Importantly, the released siRNA from hydrogels exposed to UV light could silence GFP expression to a degree that was not significantly different to the fresh siGFP (55.62%), indicating that the UV did not reduce the siRNA bioactivity.

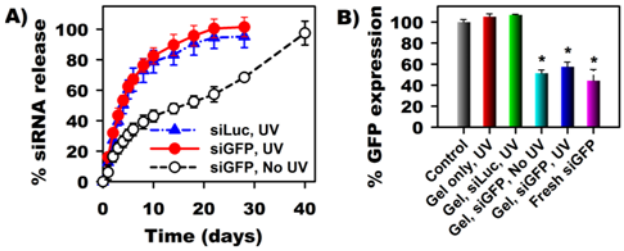

Figure 1. (A) siRNA release profiles from photolabile PEG hydrogels with and without the application of UV light, (B) % GFP expression of deGFP expressing HeLa cells after 2 days of culture with the same amount (0.5 µg siRNA/well) of fresh siGFP and release samples collected from days 18 to 22 of the release experiment. (* $p<0.01$ compared to Control).

Conclusions: A photocleavable hydrogel system capable of light-triggered siRNA release has been engineered. The released siRNA from hydrogels with and without UV exposure exhibited similar bioactivity to fresh siRNA. To the best of our knowledge, this is the first report of photolabile hydrogels for on demand delivery of genetic material. This hydrogel system may provide a promising external stimulus-based nucleic acid delivery platform for applications in disease therapeutics and tissue regeneration.

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