Nucleic acid release from carriers via physical and biological triggers

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Statement of Purpose: Hydrogels have been shown to control drug release over extended periods of time, and recent efforts involve molecular-based strategies **[1]**. The incorporation of nucleic acid constructs into hydrogels can produce novel biomaterials with programmable on-demand switches or modulatory mechanisms, with unprecedented control and sensitivity. Here we demonstrate the controlled release of nucleic acid therapeutics loaded into hydrogels via enzymatic and physical triggers. These novel biomaterials, designed using the principles of molecular biology, are expected to profoundly impact gene therapy regimes.

Methods: Custom oligonucleotides, which were partially complementary to each other and had a programmed recognition site for the restriction endonuclease BamHI, were modified with a polymerizable acrylate functionality and ^{32}P-labeled using T4 polynucleotide kinase. *In-vitro* hybridization and restriction enzyme digests were optimized and confirmed using polyacrylamide gel electrophoresis. Poly(acrylamide–co-N,N' methylene bisacrylamide-co-acrylated DNA) hydrogels were synthesized *via* redox polymerization and the unincorporated DNA was eluted by electrophoresis. Release studies of ^{32}P-labeled loaded DNA were conducted by incubating the DNA-loaded gels under physiological conditions in the presence of BamHI. Temperature was used to release the ^{32}P-labeled oligonucleotide as an alternative physical trigger. Poly(2-hydroxyethyl methacrylate-co-polyethylene glycol 600 dimethacrylate-co-acrylated DNA) hydrogels of varying crosslinking densities were triggered to release DNA by the non-specific endonuclease DNase I. The physiological significance of this platform was demonstrated by delivering a deoxyribozyme, which bore a catalytic 10-23 motif and was specific to a HIV Tat/Rev mRNA **[2, 3]**. The HIV-1 Tat/Rev RNA was synthesized by *in vitro* transcription and labeled using [5'-^{32}P]pCp and T4 RNA ligase **[4]**.

Results: *In-vitro* hybridization conditions were optimized in various concentrations of Tris buffer and water, and magnesium was determined to be critical for hybridization of the construct. About 10% of the complementary oligonucleotide self-annealed. The functional monomers used for hydrogel synthesis had no effect on hybridization efficiency. Digestion of the DNA helices was carried out by incubating the DNA helices with restriction enzyme BamHI and BamHI buffer. Incubation of the DNA double helix (without BamHI) on ice and at 37^{o}C had no effect on the duplex. BamHI buffer did not disrupt the annealed duplex. The labeled complementary oligonucleotide, when polymerized into the hydrogel without the anchoring acrylated oligonucleotide, eluted readily from the hydrogel during electrophoresis whereas acrylated DNA duplex was readily incorporated into the network. Quantification of the polymerization process *via* electrophoresis and phosphoimaging indicated 70% capture of the acrylated DNA, 25 % unincorporated acrylated DNA, and 5% ^{32}P-labeled oligonucleotide. A labeled oligonucleotide bearing a non-complementary sequence to the acrylated oligonucleotide did not get incorporated as annealing did not occur between the two strands. The release of DNA due to the penetration of restriction enzyme was shown to be highly specific, with no release in the absence of BamHI, and the presence of another endonuclease, EcoRI **[Figure 1]**. Temperature responsive release characteristics corresponded to the theoretical melting temperature of the helix (58°C). Incubation of the deoxyribozyme-loaded hydrogel and the HIV-1 Tat/Rev RNA resulted in down regulation of the gene. BamHI action on a hydrogel lacking the deoxyribozyme construct had no effect on the HIV-1 Tat/Rev RNA. Release rate of DNA from biocompatible hydrogels varied inversely with the crosslinking densities, and consequently mesh size.

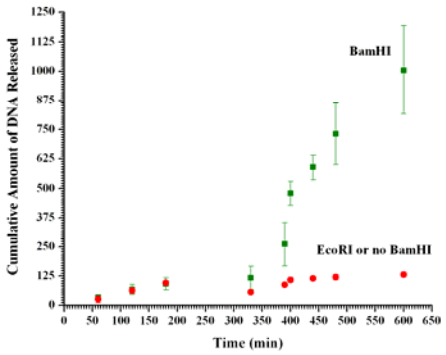

Figure 1. Tailored release of DNA by incubating the gels in BamHI at physiological conditions (■) as compared to incubating in buffer (●).

Conclusions: Substrate specific (BamHI) and substrate non-specific (DNase I) enzymes were used to trigger the release of nucleic acid therapeutics loaded into biocompatible gels. Since melting temperature is a function of G-C base pairing, sequence is an additional parameter to control release profiles. Release profiles from biocompatible gels were further tuned by varying crosslinking densities. Physiological validation of the model was demonstrated by the down regulation of a HIV-1 Tat/Rev RNA.

References:

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