Nonviral Vector Delivery from T904/Fibrinogen Hydrogels

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Statement of Purpose

Hydrogels have been widely used in tissue engineering applications due to their physical properties that closely mimic the natural extracellular matrix. In particular, gene delivery methods mediated by biomaterials such as hydrogels provide advantages over systemic delivery that include localized gene delivery, controlled and sustained release of vectors, and increased bioactivity.[1] Hydrogels composed of both synthetic and natural elements can have their properties modified by manipulation of polymer composition and cross-linking density of the hydrogel. The long-term goal of the project is to synthesize a hydrogel coating for implantable fibers that can provide both spatially and temporally controlled release of gene vectors. The objective of this study was to investigate the release kinetics of nonviral vectors from composite hydrogels formed by covalent crosslinking of a synthetic tetrafunctional block copolymer, Tetronic® T904 (T904), and a natural soluble plasma protein, fibrinogen (FgN). T904 is a member of a family of amphiphilic copolymers previously shown to support efficient delivery of plasmid DNA in vivo.[2]

Methods

Acrylated T904 (T904 ACR) was synthesized by the reaction of the terminal hydroxyl groups of T904 (BASF) with acryloyl chloride. Human fibrinogen, human alpha thrombin, and human plasmin were purchased from Enzyme Research Laboratories, South Bend, IN. The plasmid was pGFP (Promega, Madison, WI) amplified from *E. Coli* and purified with Plasmid Plus (Qiagen, Valencia, CA). JetPEI (Polyplus-transfection, France) was used as polyethyleneimine-based vector.

1. Hydrogel formation and crosslinking: Hydrogels with varying T904 ACR/FgN content were crosslinked by Michael-type addition at 1:1 acrylate:thiol ratio. FgN was first mixed with the required amount of DTT for one hour. Naked pDNA or JetPEI/pDNA complexes lyophilized using 1% sucrose as a cryoprotectant were resuspended in T904 ACR, mixed with FgN/DTT, and 90 µl samples crosslinked between glass coverslips for 1 hr at room temperature and 1 hr at 37 °C. Fully natural (20 mg/ml fibrinogen crosslinked with 5 U/ml thrombin) and fully synthetic (T904 ACR/DTT, 23% w/v T904 ACR) hydrogels were used as controls. After incubation, gels were washed with PBS for 5 minutes.

2. pGFP and JetPEI/pGFP release studies: Gels were incubated in PBS containing 100nM plasmin at 37°C for release studies to approximate physiological conditions with the buffer replaced every 48 hours.

3. DNA quantification: Amount of released pGFP was quantified utilizing the Picogreen DNA quantification assay (Invitrogen, Carlsbad, CA).

4. *In vitro* transfection assay: T904/FgN gels with encapsulated JetPEI/pGFP were incubated in PBS containing plasmin (500 nM, 24 hr). The released JetPEI/pGFP complex was used to transfect C6 glioma cells and the transfection efficiency of the complex was assessed using fluorescent microscopy.

Results

1. Plasmid and vector release from T904/FgN hydrogels: Figure 1A shows that naked pDNA released from all gel formulations had a small initial burst and slow release in the first week. The mean cumulative release in the second week was 50% to 60% for all three gel formulations. In contrast to naked pDNA, JetPEI/pDNA complexes released much slower in all gel formulations to a cumulative release from 7% to 14% after 19 days (Figure 1B).

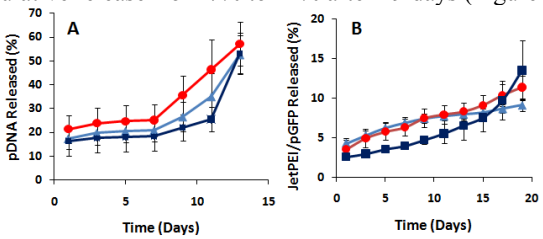

Figure 1. pDNA release from T904/FgN hydrogels (A) and JetPEI/pDNA release (B). The percentages of T904 and FgN in the hydrogels are: 10%:1.5% (▲), 10%:2% (●), and 12.5%:2% (■).

The release profiles of naked DNA from natural (fibrin), synthetic (T904) and composite T904/FgN were compared. (Figure 2). T904/FgN composite gels released pGFP faster than T904 and slower than fibrin.

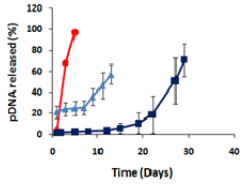

Figure 2. pDNA release from 23% T904 (▲), 2% FgN (●), and 10% T904:2% FgN (■) hydrogels.

2. Transfection of released JetPEI/pGFP : The bioactivity of released vector was assessed in C6 glioma by *in vitro* transfection (Figure 3). Lyophilized JetPEI/pGFP complexes recovered from 10% T904: 2% FgN hydrogels successfully transfected C6 cells.

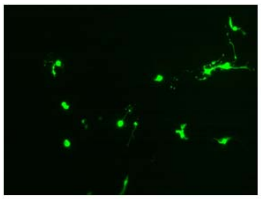

Figure 3. GFP expression in C6 cells after transfection with recovered complex.

Conclusions

We demonstrated that the slow release of pDNA from T904 hydrogels and quick release from fibrin hydrogels can be mediated by crosslinking T904 and FgN together to form a composite hydrogel. The bioactivity of recovered vector was confirmed as well with *in vitro* transfection. We are currently working on more gel compositions to provide better temporal control for released vectors.

References 1. Salvay DM. *Mol Biosyst* 2006; 2:36
2. Prokop A. *J Pharm Sci* 2002; 91:67.

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