Sustained Delivery of Novel Polymer/DNA Complexes from their Self-Assembled Injectable Gels

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Statement of Purpose: Injection of plasmid DNA in vivo using non-viral vectors generates a transient expression that decreases rapidly. The goal of this study was to develop an injectable sustained gene delivery system that can release condensed plasmid DNA over a period of time intramuscularly/ intratumorally, increasing the long term availability of gene drug, and thus maintaining the therapeutic level of expressed proteins to longer times without repeated administration. Novel block copolymers (PDEAEM-b-PEO-b-PPO-b-PEO-b-PDEAEM) of poly(diethylaminoethymethacrylate) (PDEAEM), poly(ethylene oxide) (PEO) and poly(propylene oxide) (PPO) were synthesized from Pluronic® F127 (PEO-b-PPO-b-PEO) using an ATRP reaction scheme[1]. These amphiphilic copolymers form micelles in aqueous solutions, which further self-assemble at 15wt% or higher concentrations to form elastic hydrogels reversibly at physiological temperatures[1,2]. The copolymers can be complexed with DNA in aqueous solutions at room temperatures, which when injected subcutaneously, form gels insitu on heating to body temperatures, acting as a sustained release depot for polyplexes.

Methods: Pentablock copolymers containing 20wt% PDEAEM were synthesized[1]. A pRL-CMV plasmid encoding for luciferase protein was used as a reporter gene. Transfection efficiency[3] and cytotoxicity[4] of the copolymers were tested on SKOV3 (human ovarian cancer) cell lines in serum supplemented complete growth media, using ExGen 500® (linear PEI) as a positive control. DNA was condensed in 0.5x HBS buffer, pH 7.0 with copolymers at different N/P ratios (molar ratios of nitrogens(N) of polymer to phosphates(P) of DNA). Free Pluronic® was added to the polyplex solutions in wt ratio 10:1 to pentablock copolymer to shield the surface charge of cationic polyplexes, preventing aggregation with serum proteins. Polymer concentration in the solution was made up to 15wt% by adding more pentablock or Pluronic® copolymers, and left at 4°C to form homogenous solutions. These 15wt% polyplex solutions formed elastic hydrogels at 37°C, which were then dissolved in 500µl excess buffer in 37°C shaking incubators. Samples were collected at different time points and tested for size, and stability using dynamic light scattering and agarose gel electrophoresis. Heparin (1% w/v) was added to polyplex solutions to release condensed DNA, and its concentration was measured using a picogreen fluorescence assay. Level of luciferase protein expressed in the transfected cells was measured using a Promega Renilla luciferase assay kit on a microplate luminometer, and an XTT assay was used to determine the cytotoxicity of the polyplexes.

Results/Discussion: Polyplex gels (150µl) containing 20 to 70µg plasmid dissolved over 5 to 7 days (fig 1) to release polyplexes of 150 to 200nm dia. Agarose gel

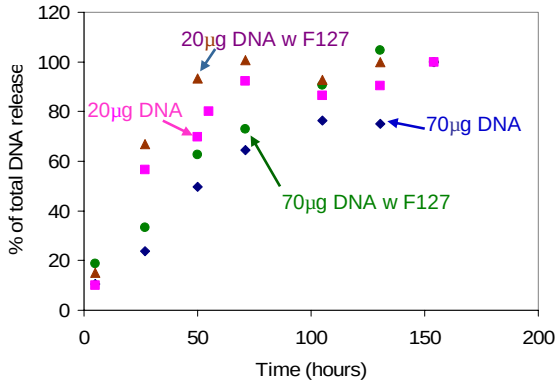

Fig 1. Sustained release of polyplexes from their gels

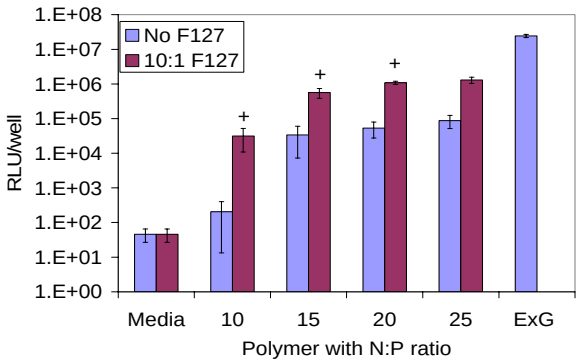

Fig 2. Luciferase expression in transfected SKOV3 cells.

electrophoresis confirmed that condensed DNA was released, and that the most of the DNA was preserved in supercoiled form. Gels containing 20µg DNA dissolved relatively faster, indicating higher amount of DNA makes strongly networked gels. Also, gels containing Pluronic® along with pentablock copolymers (to make 15wt% solution) dissolved at a faster rate than those containing no Pluronic®. Polyplexes, stabilized by free Pluronic® gave improved transfection even in complete growth media, comparable to ExGen® (fig 2). However, the cytotoxicity of copolymer's polyplexes was much lower than ExGen®. Gels will dissolve at a slower rate *in vivo* in a tissue matrix with less fluid around.

Conclusions: Thermo-reversible gels of these novel copolymer/DNA complexes release stable polyplexes over a week in vitro. Polyplexes show good transfection even in serum supplemented media. In vivo studies investigating sustained gene expression after intramuscular injection of these gels are underway.

References:

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