Surface-associated DNA presentation for sustained gene delivery with porous hyaluronic acid hydrogels

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Statement of Purpose: A promising method for promoting angiogenesis for accelerated wound healing is the controlled delivery of therapeutic genes. However, previous studies performed with DNA polyplex-loaded hyaluronic acid gels have had limited success in achieving significant levels of transfection and blood vessel development in vivo. In addition, re-transfection with bolus delivery of polyplexes is not possible due to cytotoxicity. The objective of this study is to design and characterize a tunable hyaluronic acid (HA)-based porous hydrogel system with surface-associated DNA polyplexes loaded for enhanced and sustained local gene delivery. Cells infiltrating scaffold pores will be directly exposed to surface-coated DNA, resulting in faster and increased transfection. Also, tuning DNA loading concentration, extracellular matrix protein coating of pores, and promoter design can modulate transgene expression in this system. In addition, we hypothesize that this increased and sustained transfection profile is attributed to the occurrence of re-transfection events.

Methods: HA hydrogels were formed via a Michael-addition crosslinking reaction between acrylated HA and thiolated peptide crosslinkers. A second RGD peptide for was also included to enable cell adhesion. To introduce porosity, this 4-mm diameter hydrogel was formed via the sphere templating method to produce hydrogels with 60-μm diameter pores. DNA was delivered as DNA polyplexes, which are nanoparticles composed of plasmid DNA complexed with polyethyleneimine. Polyplexes were surface coated by electrostatic association to the pore surfaces of the hydrogel by incubating the formed hydrogel in a polyplex solution. The amount of DNA loaded into the hydrogels was assessed by loading ^{32}P-labelled DNA and scintillation counting. Transfection profiles were obtained by loading DNA encoding for the Gaussia luciferase (pGLuc) gene, seeding D1 mouse mesenchymal stem cells in the hydrogels, and detecting the expressed protein with the associated assay.

Results: ^{32}P analysis of DNA-loaded porous hydrogels showed that up to 4 μg of DNA could be loaded into the scaffold. Although the DNA was electrostatically immobilized to the scaffold (neg. scaffold and pos. polyplex), minimal polyplex release was observed with only 5% of the initial loaded DNA released over 7 days. Cells seeded within the porous scaffold and with immediate contact with the polyplexes resulted in sustained transgene expression over a period of more than 30 days (Fig. 1c). In addition, adjusting the DNA loading concentration (Fig. 1c), coating the surface with varying amounts of the extracellular matrix protein fibronectin (Fig. 1d), and using plasmids with different promoters (CMV, EF1a, UbC) (Fig. 1e) were done to modulate transgene expression. To determine if sustained expression was due to re-transfection events or due to increase in space availability, D1 cells previously transfected with pGLuc and nontransfected D1 cells were seeded in porous hydrogels coated or not coated with DNA polyplexes; by seeding cells in the pores of the hydrogels, the availability of space for cell growth is dramatically increased. Previously transfected cells seeded in DNA-loaded hydrogels (+C +G) still exhibited a significant increase in transgene expression over previously non-transfected cells seeded in DNA-loaded hydrogels (-C +G) (Fig. 1f).

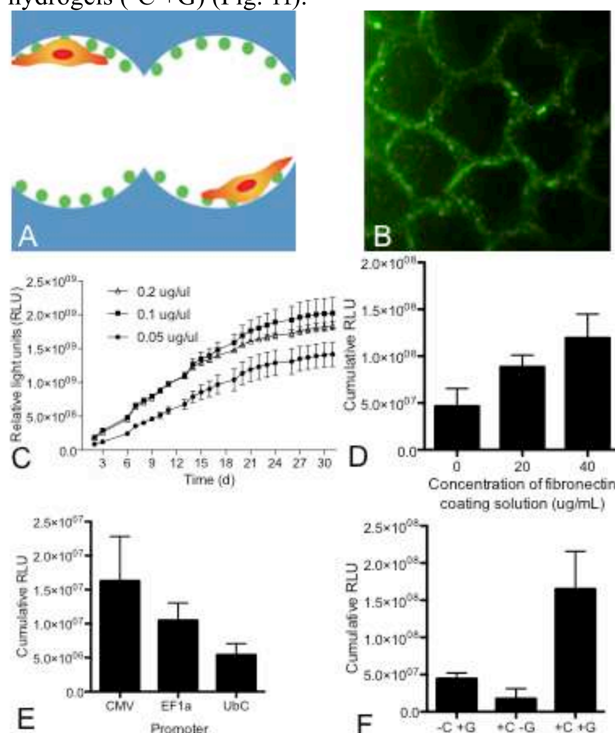

Figure 1: (a) Schematic of porous HA gel with surface coated polyplexes and seeded with cells; (b) Stained DNA polyplexes surface-coated in porous HA hydrogel; (c) Hydrogels coated in varying DNA polyplex concentrations were seeded with D1 cells and cultured for 31 d to assess transgene expression profile; (d) DNA-loaded hydrogels were coated at different concentrations of fibronectin and seeded with D1 cells over 7 d; (e) Promoter effect on transgene expression over 16 d; (f) Previously transfected (+C) and nontransfected (-C) cells were seeded in porous hydrogels coated (+G) or not coated with DNA (-G) and cultured over 7 d.

Conclusions: After DNA loading by surface coating, there was minimal basal DNA release over time, implying that this may serve as a robust system for long-term DNA availability and expression. Indeed, sustained expression was seen over thirty days of culture and could be enhanced by increasing DNA loading, fibronectin coating density, or by changing promoter design. Finally, the sustained expression observed was due to multiple re-transfection events, not increased space availability, as already-transfected cells seeded in DNA-loaded gels exhibited enhanced expression over non-transfected cells seeded in gels with DNA. This system is a promising means of sustained gene delivery for various biomedical applications without the need for genomic integration via viral methods, which have innate safety concerns.