DNA Delivery in Hyaluronic Acid Hydrogels

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Statement of Purpose:

Scaffolds capable of controlled efficient gene delivery are powerful tools for tissue engineering, with numerous applications. The scaffold creates and maintains a space for tissue formation, and provides a support for cell adhesion. Gene delivery represents a versatile approach to promote the specific cellular processes that lead to tissue formation. Transfected cells will then be produce tissue inductive proteins that are secreted into the local microenvironment.

This study develops photopolymerizable hydrogels capable of controlled DNA delivery and investigates transgene expression in vitro. Photopolymerization would enable gels to be formed in situ, and must allow for DNA encapsulation without significantly affecting the activity of the entrapped DNA. Hydrogels will be formed from hyaluronic acid (HA), which is a naturally occurring biopolymer with roles in wound healing and inflammation.[1,2] HA has minimal non-specific protein adhesion, is biodegradable, and has functional groups available for chemically modification.[1,2] Naked plasmid and plasmid complexed with a positively charged polymer, polyethylenimine (PEI) are entrapped within the polymer. The incorporation efficiency, release rates, and transfection efficiency are investigated based on the hydrogel properties and crosslinking conditions.

Methods:

In order to form photocrosslinkable hydrogels, hyaluronic acid was chemically modified by replacing a percentage of carboxyl groups with acrylate groups using a two-reaction process. The first reaction involves coupling adipic acid dihydrazide to carboxyl groups using 1-ethyl-3-[3-dimethylaminopropyl]carbodiimide hydrochloride (EDC), in the presence of N-hydroxysuccinimide (sulfo-NHS), which converts carboxyl groups to amine-reactive sulfo-NHS esters that react with AAD. In the second reaction, available amines on AAD are reacted with NHS-PEG-Acryl. The percent completion of reaction steps one and two was determined by a trinitrobenzene sulfonic acid (TNBS) assay[1] and proton NMR[2], respectively. The acrylated HA (HA-Acryl) was crosslinked with PEG-Acryl to create stable hydrogels. Hydrogels are fabricated using UV-radiation (365 nm) to polymerize solutions of 1% HA-Acryl, and either 2% or 4% PEG-Acryl. All solutions contain 1% of the photoinitiator Igracure 2959.

Encapsulation of DNA plasmids, cells, and/or DNA/PEI complexes was performed by mixing them with the HA solutions prior to crosslinking**.** DNA release was characterized using Hoechst staining or P^{32} labeled plasmid. HA degradation was monitored through the carbazole reaction by UV-Vis spectroscopy. Transfection efficiency was determined using a GFP or ß-galactosidase plasmid to determine the number of transfected cells, and Hoechst dye to identify the total cell number.

Results / Discussion:

Characterization of HA-AAD using a TNBS assay indicated that the percent of carboxyl groups reacted with AAD was approximately 35%. The addition of the acryl groups was characterized by proton NMR, and indicated acrylation of approximately 11% of the available hydrazide groups.

Hydrogels were subsequently formed by crosslinking HA-Acryl with PEG Acryl. Solutions containing 1% HA-Acryl, 1% photoinitiator and PEG-Acryl varying from 2-4% crosslinked using UV-radiation at 365 nm were suitable for fabricating stable hydrogels. Varying the concentration of the PEG-Acryl allowed for control over crosslink density of the hydrogel. As expected, HA degradation significantly decreased with increased crosslink densities.

Naked plasmid and DNA/PEI complexes were stably incorporated into the hydrogel, and exhibited sustained release over at least 7 days. Naked plasmid encapsulated and released from the hydrogel retained its supercoiled conformation. Inducing hydrogel degradation with hyaluronidase led to increase rates of release, suggesting that the crosslink density can be employed to regulate the release rate. Manipulating the crosslink density to control the release rate may be important for regulating the local concentration of DNA.

MCF-7 cells could be encapsulated within the hydrogels with minimal toxicity, and were efficiently transfected by entrapped PEI/DNA complexes. Conditions for hydrogel fabrication are mild, and maintained greater than 95% cell viability. Transfection efficiencies to were greater than 80% for photocrosslinked HA hydrogels with cells encapsulated in the presence of DNA/PEI complexes, indicating that entrapped complexes maintain their activity.

Conclusions:

We have created photopolymerizable hydrogels by modifying hyaluronic acid with AAD and acryl groups, which can be crosslinked with a 4-arm PEG-Acryl. The technique for creating the HA-Acryl/PEG-Acryl hydrogels employs conditions mild enough for the encapsulation of cells without affecting cell viability, and DNA without compromising integrity or activity. Crosslink density of the hydrogels can be controlled via the percentage of PEG-Acryl incorporated within the gel, and can regulate the degradation rate of the hydrogel and the release rate of DNA. Entrapped complexes are active leading to high transfection efficiencies in vitro. HA is a promising biomaterials due its material and chemical properties, and hydrogel based gene delivery has great potential in tissue engineering applications.

References:

1. Segura, T. et al.: *Biomaterials* **2005**, 26, 359-37.
2. Leach, J. et al.: *Biotechnol. Bioeng.* **2003**, 82, 578-589.