Development of Amphiphilic Calcium Alginate Nanoparticles for Controlled Gene Delivery

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Statement of Purpose: Bolus delivery, or burst release, genetic vaccines often does not elicit an adaptive immune response. Temporal control of the repetitive delivery of genetic material may be an effective means of generating an adaptive immune response. An innocuous naturally derived polysaccharide, alginate, was chosen for the encapsulation and delivery of DNA vaccines. The hydrolysis and degradation rate of the polymer can be modified via hydrophobic chemical modification. Thus, the diffusion and release rate of the genetic material may be controlled and optimized. The overall goal of this project is to control the temporal delivery a DNA vaccine for the efficient transfection of antigen-presenting cells. Our objective here is to fabricate and characterize novel amphiphilic calcium alginate nanoparticles for the packaging and controlled release of cationic polymer condensed (polyplexed) DNA.

Methods: Sodium alginate (Sigma) was purified before use.[1] Briefly, a sodium alginate solution was mixed with activated charcoal granules. The solution was filtered and pH was adjusted to 4.0 with 1N HCl. The purified alginic acid (AA) was precipitated with an excess of EtOH, dried at room temperature, dialyzed against DI water, and lyophilized. Aqueous solutions of AA and hexadecyltrimethylammonium bromide (CTAB) were mixed together to form the precipitate AA-CTA. To form methacrylated AA (AA-MA), methacrylic anhydride was added to an AA-CTA dimethyl sulfoxide solution and mixed at 4°C for 24 h.[2] CTA salts were removed via hydrolysis with 0.2M NaCl. AA-MA was dialyzed and lyophilized. To form AA-C_{12}, dodecyl bromide was added to an AA-CTA solution and mixed at room temperature for 24 h.[3] Removal of CTA salts and purification of the product was similar to the methods described above. Fourier Transform infrared (FTIR) was used to verify the formation of AA-CTA, subsequent hydrolysis of AA-CTA, and AA-C_{12}. Proton nuclear magnetic resonance (^{1}H-NMR) was used to verify and quantify the synthesis of AA-MA. Polyethylenimine (PEI) and DNA (pCMV-luciferase) solutions were mixed to form polyplexes (N:P=10). An oil/water emulsion technique was used to fabricate polyplex encapsulated alginate particles.[4] Alginate (Na-AA, AA, AA-MA, AA-C_{12}) and polyplex solutions were mixed together then blended into a 5% Span 80 oil solution. A 90mM calcium chloride solution was then added. Unadsorbed DNA was quantified by centrifuging the reaction solution and analyzing the supernatant via UV spectroscopy at 260 nm. Encapsulation efficiency (EE) was calculated as the measured DNA content in the particles divided by the amount of DNA added to the reaction x100. The shape of the particles was assessed via optical microscopy. Average diameter and zeta potential of the particles were assessed using a zeta-sizer (Malvern Instruments). *In vitro* DNA release profiles were determined by placing the particles in phosphate buffered saline supplemented with 0.05% polyvinyl alcohol at 37°C under mild agitation. The *in vitro* release products were analyzed via UV spectroscopy.

Results: FTIR confirmed the formation of AA-CTA and removal of the CTA salt via hydrolysis. FTIR also confirmed the synthesis of AA-C_{12} through the introduction of methyl, methylene and ester peaks. ^{1}H-NMR confirmed the formation of AA-MA through the introduction of methyl peak and methylene peaks; the degree of substitution was 1.6%. Alginate particles were spherical and uniform in shape. EE, particle diameter, and zeta potential measurements are shown in **Table 1**. *In vitro* DNA release data is shown in **Fig.1**.

Table 1. Characterization results for condensed DNA (polyplex) DNA encapsulated alginate nanoparticles.

	EE (%)	Diameter (nm)	Zeta Potential (mV)
Polyplex	n.a.	128 ± 3	42.3 ± 2.86
Na-AA	95.9	740 ± 89	-38.5 ± 0.71
AA	94.6	704 ± 70	0.2 ± 0.01
AA-MA	94.7	596 ± 61	0.4 ± 0.35
AA-C_{12}	89.1	234 ± 20	-48.0 ± 4.24

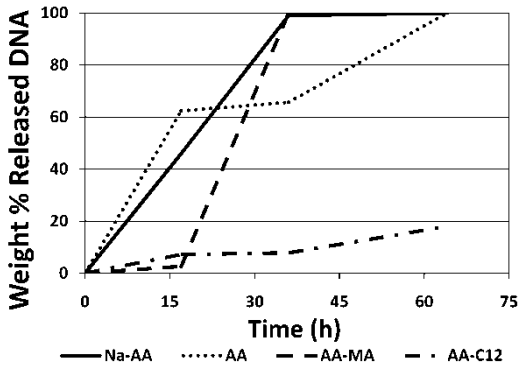

Fig.1. *In vitro* release of DNA from alginate nanoparticles.

Conclusions: A method to prepare amphiphilic alginate nanoparticles was developed to yield small, uniform particles. PEI-complexed DNA was encapsulated in amphiphilic alginate nanoparticles with high yields. The hydrophobic domains on the modified alginate afford good retention of the polyplexes by retarding hydrolysis of the nanoparticles. The temporal release of polyplexes and diameter of the nanoparticles may be further optimized by varying the degree of substitution of AA-MA and AA-C_{12} polymers, thus controlling the diffusion and release rate of the polyplexes. The high EE of DNA from amphiphilic alginate nanoparticles is encouraging for application in the field of gene therapy and vaccine delivery. Studies are in progress to evaluate cell-particle interactions and transfection ability.

References: 1. Dussealt J. *et al.* J Biomed Mat Res. 2005;76A:243-251; 2. Soon-Shiong P. *et al.* US Patent No. 5846530, 1998; 3. Pelletier S. et al. Carbohydrate Polymers. 2000;43:343-349; 4. Jay SM. and Saltzman WM. Journal of Controlled Release. 2009;134:26-34.