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The effect of swelling and cationic character on gene transfection by pH-sensitive nanocarriers

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ABSTRACT

We synthesized a series of pH-sensitive vehicles, composed of dimethylaminoethyl methacrylate (DMAEMA) and 2-hydroxyethyl methacrylate (HEMA), to optimize the triggered release of DNA for gene transfection. The purpose of this study was to assess the role of swelling and cationic character independently on transfection; both of which may affect DNA release. Gene transfection was performed by delivering plasmid DNA (pDNA) encoding for luciferase. DNA release was controlled via volumetric swelling by regulating the endosomal pH as a result of inhibiting V ATPases using bafilomycin A1. Increasing the cationic character from 10 to 30 mol% DMAEMA did not increase transfection when swelling was inhibited. Transfection was significantly affected by the rate of pDNA release. pH-sensitive nanocarriers were also compared to vehicles comprised of polyethyleneimine (PEI), dioleoyl trimmonium propane (DOTAP), and poly(lactic-co-glycolic acid) (PLGA, 50:50). pDNA encapsulating DMAEMA/HEMA nanoparticles and PEI/pDNA complexes had reduced transfection when V ATPases were inhibited, whereas pDNA encapsulating PLGA nanoparticles showed no endosomal pH dependence. DMAEMA/HEMA nanoparticles cross-linked with 3 mol% tetraethylene glycol dimethacrylate (TEGDMA) reported equivalent or greater gene transfection relative to the nanocarriers tested at 24 and 48 h.

1. Introduction

Therapeutic gene delivery is based on the concept that recombinant DNA and RNA interference technologies can be used to regulate disease at the molecular level. Nonetheless, treatment of human diseases by genetic material instead of drugs has been limited by effective delivery without cytotoxicity. Nonviral gene delivery vehicles have encapsulated or condensed pDNA with cationic polymers [1–5], degradable polymers [6], or lipids [7], where the pDNA is subsequently released using the low endosomal pH [8]. While it is widely accepted that pH facilitates endosomal delivery, the consequences of vehicle cationic character and the rate of DNA release are poorly understood.

Endocytosis generally occurs by engulfing molecules or therapeutic vehicles by the plasma membrane. Plasma membrane invaginations evolve into endosomes that become lysosomes, acidic compartments responsible for degrading foreign agents. Therapeutic agents (i.e. pDNA) are delivered to the cytoplasm by disrupting endosomes (Fig. 1).

Endosomes undergo acidification by ion pumps. Most primary ion pumps use the energy provided by the hydrolysis of adenosine triphosphate (ATP) to energize ion-transport processes across the cell membrane. V ATPases [9] (i.e. H^+ ATPases), found on endosomal membranes, are responsible for endosome acidification [10,11]. Bafilomycin A1, a proton pump inhibitor, has been used to inhibit endosome acidification. The average pH of the endosome was ~5.5 in the absence and 7.4 in the presence of 200 nM bafilomycin A1 [10]. Bafilomycin A1 has been employed previously to assess endosomal gene delivery [12].

Endocytosed vehicles deliver pDNA by destabilizing the endosomal envelope [13] or buffering against lysosomal degradation by the "proton sponge" effect [2]. Dioleoyl trimethyl ammonium propane (DOTAP) [14] and polyethylenimine (PEI) [15] have been used to condense DNA; however, they both induce cytotoxic effects [15,16]. Peptides [17,18] and fusogenic liposomes [19], activated by low pH, have been designed to induce endosome disruption. pDNA encapsulating poly(lactic-co-glycolic acid) (PLGA, 50:50) nanoparticles have been investigated for gene delivery [20,21], where PLGA degrades by acid hydrolysis. Release of pDNA from the endosome to the cytoplasm is important in facilitating gene transfer.

Endosomal delivery may also depend on the vehicles cationic character. The N/P ratio, the ratio of polycationic polymers or

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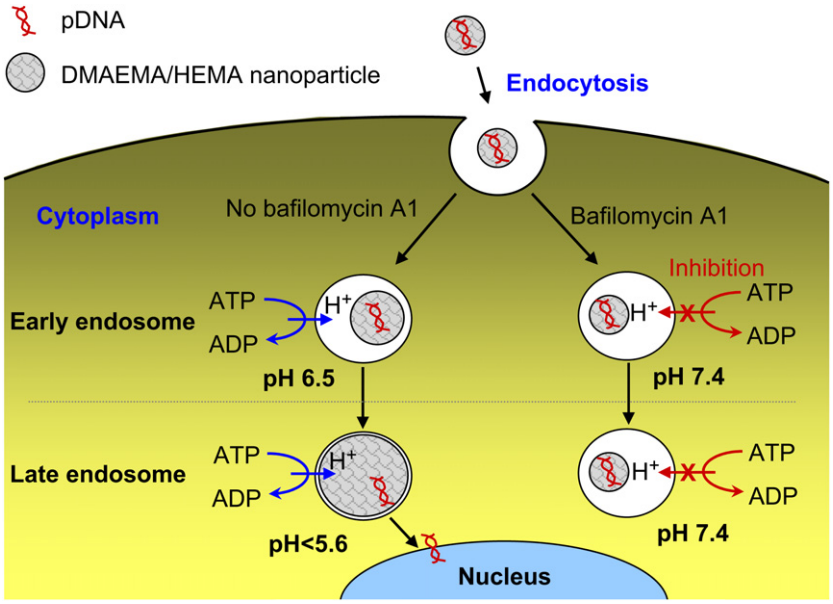

Fig. 1. Schematic representation of pH-sensitive DMAEMA/HEMA nanoparticle-mediated gene transfection with and without bafilomycin A1 as a V ATPase inhibitor.

cationic lipids (with N amine groups) to pDNA (with P phosphate groups), is often used to optimize gene delivery. A high N/P ratio may inhibit pDNA release whereas a low N/P ratio may inefficiently complex the pDNA [22]. In addition, increased cationic character has been linked to increased cell uptake [23]. The contribution of the cationic charge to gene delivery is multifold.

Recently, we synthesized a series of nanoparticles with tunable pH-sensitivity to optimize gene delivery [24]. These particles trigger the release of pDNA in response to changes in pH. Although particles had distinct differences in volumetric swelling as a function of pH, transfection of HeLa cells by pH-sensitive nanoparticles was not dramatically affected by increasing the amount of the pH-sensitive monomer dimethylaminoethyl methacrylate (DMAEMA). Increasing the DMAEMA content simultaneously increased the cationic character and swelling ratio. Therefore, the contribution of pH-induced swelling and cationic character on transfection was unclear.

In this paper, we investigated the role of vehicle swelling and cationicity independently on transfection. Both the extent of swelling and the electrostatic interactions between the cationic DMAEMA and anionic pDNA may affect the rate of pDNA release. We controlled volumetric swelling by altering the endosomal pH as a result of inhibiting V ATPases using bafilomycin A1. Gene transfection was performed by delivering pDNA encoding for luciferase to HeLa, a human cervical cancer cell line, and HEK293, a human embryonic kidney cell line. pDNA encapsulating, pH-sensitive vehicles were benchmarked against naked pDNA, PEI/pDNA complexes, DOTAP/pDNA complexes, and pDNA encapsulating PLGA nanoparticles.

2. Material and methods

2.1. Materials

The monomer dimethylaminoethyl methacrylate (DMAEMA) and comonomer 2-hydroxyethyl methacrylate (HEMA) were purchased from Acros (Morris Plains, NJ, USA). The cross-linker tetraethylene glycol dimethacrylate (TEGDMA) was obtained from Fluka (St. Louis, MO, USA). The bafilomycin A1, polyethyleneimine (PEI, 750 kDa), chloroform, ammonium persulfate, and sodium metabisulphite were purchased from Sigma (St. Louis, MO, USA). 1,2-dioleoyl-3-trimethyl-ammonium-propane (DOTAP) was purchased from Avanti Polar Lipids (Alabaster, Alabama, USA). Pluronic F68 was kindly provided from BASF Corporation (Mount Olive, NJ, USA). Poly(lactide-co-glycolide) (PLGA, 50:50, MW 17000–22000) and polyvinyl alcohol (PVA, MW ~25000) were purchased from Polysciences, Inc. (Warrington, PA, USA) and dichloromethane was purchased from Mallinckrodt Baker, Inc. (Phillipsburg, NJ, USA). gWiz™ high-expression luciferase vector was purchased from Aldevron (South Fargo, ND, USA) for gene transfection. All cell culture media were purchased from Invitrogen (Carlsbad, CA, USA). All materials were used without further purification. Deionized water (18.2 MΩ) was obtained from a Milli-Q purification system (Millipore Corp., Billerica, MA, USA).

2.2. Nanoparticles preparation

pDNA encapsulating, pH-sensitive DMAEMA/HEMA nanoparticles were obtained by adding 100 μL of a DMAEMA/HEMA solution (10/90, 20/80, and 30/70, mol/mol) with 3, 6, and 9 mol% TEGDMA cross-linker, containing 10 μg DNA, to 10 mL of deionized water containing Pluronic F68 (150 mg) and TEGDMA (3 mol%). Aqueous solutions of ammonium persulfate (0.5% w/v) and sodium metabisulphite (0.25% w/v) were added as initiators. The solution was immediately sonicated (200 W, 20 kHz; Digital sonifier 250, Branson Ultrasonics Corp., Danbury, CT, USA) in a laminar flow hood over an ice bath for 10 min (8 s on and 4 s off). The polymerization process was carried out at room temperature for 3 h. DMAEMA/HEMA nanoparticles were collected by high-speed centrifugation at 39000 × g for 20 min (Sorvall RC26 Plus, SA-600 rotor; Thermo Fisher Scientific Inc., Waltham, MA, USA). The particles were washed three times and collected with pH 7.4 phosphate buffer to remove residual surfactant and initiators.

PLGA nanoparticles encapsulating luciferase pDNA were prepared by a water-oil-water (W/O/W) emulsion and then solvent evaporation method. Briefly, 10 μg of pDNA (5 mg/mL in water) was directly added to 20 μL dichloromethane containing 50 mg PLGA and emulsified using a microtip probe sonicator for 10 s. The emulsion was added to 2 mL of 1% (w/v) polyvinyl alcohol solution and sonicated for 30 s at 200 W output in an ice bath to form the W/O/W emulsion. The final double emulsion was agitated using a magnetic stirrer for 3 h to remove dichloromethane completely. PLGA nanoparticles encapsulating pDNA were isolated by high-speed centrifugation at 39,000 × g for 20 min and washed three times with deionized water.

PEI/pDNA and DOTAP/pDNA complexes were prepared as described previously [2,7]. Briefly, 10 μg of pDNA and the desired amount of PEI as indicated by the N/P ratio (MW of PLL = 750 kDa, $N/P = 6$) were diluted into 50 μL of 150 nM NaCl and vortexed gently and spun down briefly. For DOTAP/pDNA complexes, dried DOTAP was rehydrated with distilled water at a final concentration of 1 mM. 10 μg of pDNA and the desired amount of DOTAP (MW of DOTAP = 698.55, $N/P = 2$) were added to deionized water in a final volume of 100 μL. The solutions were incubated for 20 min at room temperature. The N/P ratios were chosen to give high transfection based on previous reports [15,25,26].