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Composition of PLGA and PEI/DNA nanoparticles improves ultrasound-mediated gene delivery in solid tumors *in vivo*

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Abstract

The goal of this study was to enhance gene delivery and tumor cell transfection *in vivo* by using a combination of ultrasonication with complex nanoparticles consisting of two types of nanoparticles: PEI/DNA β-gal plasmid with highly positive *zeta*-potential and air-filled poly (lactic-co-glycolic acid) (PLGA) particles (with negative *zeta*-potential) manufactured in our laboratory. The PLGA/PEI/DNA nanoparticles were a colloid with positive *zeta*-potential and injected i.v. in nude mice with DU145 human prostate tumors. We found that the combination of PLGA/PEI/DNA nanoparticles with ultrasonication substantially enhanced tumor cell transfection *in vivo*. The overexpression of β-gal gene was evaluated histochemically and by Western blot analysis. At least an 8-fold increase of the cell transfection efficacy was obtained in irradiated tumors compared to non-irradiated controls, while little to no cell death was produced by ultrasonication.

Keywords: PLGA nanoparticles; PEI; Gene delivery; Ultrasound; Tumor

1. Introduction

Gene delivery into tumor cells *in vivo* is still problematic because gene targeting vectors have to overcome chemical and structural barriers to reach tumor cells. Therefore, non-viral gene transfer has low efficiency *in vivo* and transfection with intravenously administered plasmid DNA is difficult [1]. Various gene delivery systems based on nanoparticles have been developed and different polymers have been tested as gene delivery agents [1,2].

Polyethylenimine (PEI) is one of the most efficient non-targeted polycations used for gene delivery [3–5]. PEI and derivatives of PEI are generally known as

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efficient dispersants and cationic flocculants, which are believed to act with negatively charged colloids. PEIs exhibit a high positive charge density in aqueous solutions and are considered to be promising candidates for delivery of negatively charged molecules of DNA even for *in vivo* applications [6]. The relatively high efficiency of PEIs is believed to stem from a high amine density and buffering capacity [7]. This polymer forms complexes with DNA as a result of cooperative electrostatic interactions between the ammonium groups of the polycation (PEI) and phosphate groups of the DNA [8].

In newer compositions, biodegradable and biocompatible polymer poly (lactic-co-glycolic acid) (PLGA) particles are used together with derivatives of PEI [6,9]. PLGA nanoparticles have opposite (negative) charge that results in poor transport through mucosal barriers [10]. To enhance permeability these particles can be modified by a cationic agent like PEI. It has been shown that PLGA nanoparticles are able to bear PEI on their surface [6]. Moreover, encapsulation of β-galactosidase plasmids condensed by PEI in a PLGA sponge scaffold has been shown to retain DNA in tissues and enhance gene transfer [9,11].

Ultrasound is being used for gene delivery *in vitro* and *in vivo* [1,12–16]. Recently, we demonstrated that interaction of ultrasound with nanoparticles may enhance drug and gene delivery in tumor cells *in vivo* [13] because it alters properties of tumor vasculature and the cell membrane. This interaction induces non-thermal effects including mechanical membrane stretching due to nanoparticle oscillations, radiation force, and acoustic streaming which all may contribute to the enhancement of drug and gene delivery [13,14]. The nanoparticles can be accumulated in tumors using passive or active delivery. It is well established that nanoparticles injected into the bloodstream can selectively accumulate in tumor vasculature by the passive (without antibodies directed against tumor blood vessels) delivery due to the enhanced permeability and retention (EPR) effect [15,17]. Several groups of investigators have studied this effect and demonstrated accumulation of nanoparticles in the pores of the tumor blood vessels. For instance, Hobbs et al. demonstrated accumulation of nanoparticles in a variety of tumor types in nude mice and found that the tumor vasculature pore cut off is 200–1200 nm depending on the tumor type [17]. The active delivery uses targeting molecules attached to the nanoparticles [12,15–18]. Moreover, it was demonstrated recently that ultrasonication produced a significant enhancement of antitumor activity of some anticancer drugs [19,20].

In our current study, we used combination of the chemical (PLGA/PEI/DNA nanoparticles) and physical (ultrasonication) approaches for improving the gene transfer efficiency *in vivo* at intravenous administration. The proposed approach to combine ultrasonication with PLGA/PEI/DNA nanoparticles was evaluated in a nude mouse model in this study. Our results indicate that interaction between the complex nanoparticles with ultrasonication opens new possibilities for *in vivo* gene delivery.

2. Materials and methods

We manufactured PLGA particles, isolated their nanometer fraction, and also took advantage of the fact that PLGA nanoparticles possess adjuvant properties for cell targeting [21]. We prepared PEI/DNA nanoparticles, loaded them on the PLGA nanoparticles, and evaluated their properties. The obtained complex PLGA/PEI/DNA had properties which enabled efficient application of the complex nanoparticles *in vivo*.

2.1. Materials

The chemicals used in this study were purchased from the following companies: Polymer poly (DL-lactide-co-glycolide) (PLGA) 50:50 Resomer RG 504 H, (MW approx. 48,000 Da) – from Boehringer Ingelheim Pharma KG (Petersburg, VA, USA); poly (vinyl alcohol) (PVA), 88 mol%, (MW of 25,000 Da) – from Polysciences, Inc. (Warrington, PA, USA); laboratory grade (1R)-(+)-camphor, Trizma base – from Sigma Chemical Co. (St. Louis, MO, USA); Ammonium carbonate – from J.T. Baker (Mallinckrodt Baker, Inc., Phillipsburg, NJ, USA); methylene chloride, Isopropanol, Hexane of analytical grade – from Fisher Scientific (Fair Lawn, NJ, USA); LacZ reporter plasmid pCMVβ – from BD Bioscience (San Diego, CA, USA); *in vivo* jetPEI™ – from Bridge transfection (Portsmouth, NH, USA); and β-galactosidase reporter gene staining kit – from Sigma (St. Louis, MO, USA).

2.2. PLGA nanoparticle fabrication and characterization

The PLGA nanoparticles were obtained by sequential filtration of PLGA basic suspension which was prepared by double emulsion solvent evaporation technique reported by El-Sherif and Wheatly [22] with some modifications. Particle size depends on final concentration of PLGA and PVA [23] (1.25% and 2.5% in our experiments). Briefly, camphor (0.05 g) and then PLGA (0.5 g) were dissolved in 20 ml of methylene chloride. After adding of 1.0 ml of 4% ammonium carbonate, the polymer solution was ready for the first water-in-oil emulsion generation. We used a 5-