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Efficient gene delivery to primary human retinal pigment epithelial cells: The innate and acquired properties of vectors

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ABSTRACT

The purpose of this study is designing non-viral gene delivery vectors for transfection of the primary human retinal pigment epithelial cells (RPE). In the design process of gene delivery vectors, considering physicochemical properties of vectors alone does not seem to be enough since they interact with constituents of the surrounding environment and hence gain new characteristics. Moreover, due to these interactions, their cargo can be released untimely or undergo degradation before reaching to the target cells. Further, the characteristics of cells itself can also influence the transfection efficacy. For example, the non-dividing property of RPE cells can impede the transfection efficiency which in most studies was ignored by using immortal cell lines. In this study, vectors with different characteristics differing in mixing orders of pDNA, PEI polymer, and PLGA/PEI or PLGA nanoparticles were prepared and characterized. Then, their characteristics and efficacy in gene delivery to RPE cells in the presence of vitreous or fetal bovine serum (FBS) were evaluated. All formulations showed no cytotoxicity and were able to protect pDNA from premature release and degradation in extracellular media. Also, the adsorption of vitreous or serum proteins onto the surface of vectors changed their properties and hence cellular uptake and transfection efficacy.

1. Introduction

The retinal pigment epithelial (RPE) cells are a monolayer of pigmented and bipolar epithelial cells, which form a part of the retina. They are located between photoreceptors and choriocapillaris. Having various functions, the RPE cells are crucial for visual functions and homeostasis of the retina. Dysfunction of RPE cells is the leading cause of many hereditary and acquired diseases, including age-related macular degeneration (AMD) (Strauss, 2005). Therefore, RPE cells could be the target of therapy in many ocular diseases.

It is well known that gene imbalance occurs in pathologic conditions such as ocular diseases (Kawa and Machalinska, 2014; Wang et al., 2012). Restoring gene expression by gene therapy can be considered as a therapeutic approach. Although eye is an accessible organ for gene delivery, there are some barriers to delivery of therapeutic agents to ocular cells, especially the cells at posterior segment of the eye. These barriers hamper vector delivery from blood circulation or even from local administration to posterior part of the eye. Intravitreal injection is one of the most appropriate approaches for drug delivery to the posterior part of the eye. Topical and systemic drug delivery routes suffer from poor drug availability, and the eye is more prone to damage on some other routes like the transscleral one (Shah et al., 2010). In intravitreal delivery, therapeutic systems are injected into the vitreous. Vitreous gel is a three-dimensional network composed of

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glycosaminoglycan (namely hyaluronan), collagen and some other proteins, including serum proteins (Bishop, 1996). Having negative charge, vitreous can affect the distribution of positively charged particles (Pitkänen et al., 2003; Xu et al., 2013; Koo et al., 2012; Kim et al., 2009). It can also release the genetic agent from vector before reaching the target cell or destroy the genetic material by its nucleases (Peeters et al., 2005; Pitkänen et al., 2003). Adsorption of vitreous proteins onto the surface of vectors can also change their surface characteristics and influence their destination. In addition, the pore size of the vitreous network can limit the permeation and movement of the vector (Pitkänen et al., 2003; Xu et al., 2013; Sakurai et al., 2001). So, considering these phenomena is essential for vector design and optimization of physicochemical characteristics of the vector to achieve efficient gene delivery.

Many attempts have been made for in vitro gene delivery to RPE cells (primary cells or cell lines) by polymeric and liposomal vectors (Männistö et al., 2002, 2005; Peeters et al., 2007; Chaum et al., 2000; Sunshine et al., 2012; Abul-Hassan et al., 2000; Bejjani et al., 2005; Jayaraman et al., 2012; Mannermaa et al., 2005; Liu et al., 2011; Peng et al., 2011). It was shown that liposomes are not as efficient as polymeric vectors because they could not protect the gene from degradation in the lysosomes. Given its proton sponge-like effect, PEI can escape from lysosomal degradation and would thus be an appropriate choice, even though its toxicity must be considered. A useful solution to reduce the toxicity of PEI is to combine it with other polymers (Peng et al., 2011). Poly (D, L-lactide-co-glycolide) (PLGA) is a biodegradable and biocompatible polymer, which has been widely used for gene and drug delivery. It was shown that PLGA and PLA polymers had little toxicity for RPE cells (Bejjani et al., 2005). Incorporation of hydrophilic genetic material with PLGA could occur through loading in the core compartment of PLGA nanoparticles during PLGA NP synthesis (Cun et al., 2010) or by surface adsorption through applying positively charged polymers (Da Shau et al., 2012). The latter approach is less destructive for the integrity of the genetic material, and the loading capacity can be higher in comparison with the former one. It must also be taken into account that nutrient requirement, physiology, doubling time and receptor distribution is different among different cell types, which could impact the cell uptake (Izumisawa et al., 2011; Douglas et al., 2008; Assanga and Lujan, 2013). Primary RPE cells and cell lines (ARPE 19 and D407) as well as hESC-REP are available for in vitro studies on RPE cell. Therefore, the best candidates for uptake studies would be the primary ones because of high similarity to their cells of origin.

Despite enormous studies on gene or drug delivery to the eye cells, there have been limited studies considering the interaction of vectors and vitreous as well as its effect on transfection and gene expression.

In this study, we aimed to assess the transfection efficacy of polymeric nanoparticles once interact with their surrounding environment and elucidate the factors governing an optimized gene delivery to primary RPE cells. For this purpose, PLGA and PEI-based vectors were used. We have prepared three different arrangements of PEI polymer, pDNA, and PLGA or PLGA/PEI nanoparticles considering the toxicity and optimum polymer to pDNA ratio, as follow: formulation I [(PLGA/PEI)/pDNA/PEI], formulation II [(PLGA/PEI)/(pDNA/PEI)], which differs from formulation I in mixing order and the formulation III [PLGA/(pDNA/PEI)]. The surface characteristics changes in contact with various media (cell culture media with and without serum and vitreous) and their impact on cellular uptake and gene expression were evaluated.

2. Materials

Acid terminated PLGA (Purasorb PDLG 5004A, Mw = 44 kDa, DL-lactide/Glycolide molar ratio 50/50) was purchased from Corbion Purac (The Netherlands). PEI (branched, 25 kDa), PVA (30–50 kDa, 87–89% hydrolyzed), coumarin-6 and Thiazolyl blue tetrazolium bromide (MTT) were obtained from Aldrich (Germany). Fetal bovine serum (FBS), Dulbecco Modified Eagle's Medium (DMEM): F-12 (1:1) nutrient mixture (Ham) were bought from Gibco (Belgium). LysoTracker® Red DND-99 was purchased from Life Technologies (USA), and 40% Acrylamide/Bis solution (29:1) was obtained from Bio-Rad (USA).

3. Methods

3.1. Preparation of PLGA and PLGA/PEI nanoparticles

PLGA and PLGA/PEI nanoparticles were obtained by emulsion–diffusion–evaporation (o/w) method as formerly explained by Da Shau et al. with some modifications as follows (Da Shau et al., 2012). The organic phase was prepared by dissolving PLGA in ethyl acetate (a partially miscible solvent with water) at a concentration of 0.25% W/V. The aqueous phase was prepared by dissolving PVA (2% W/V) as emulsifier with deionized water. In the case of PLGA/PEI nanoparticle preparation, PEI (with regard to PEI/PLGA = 6/10, W/W) was also added to this phase. At the next step, O/W emulsion (at a phase ratio (V/V) of 1/8) was prepared by dropwise adding of organic phase to aqueous phase under stirring. The foregoing emulsion was kept stirring for about 45 min for cross diffusion of both solvents. After saturation of phases, homogenizing was performed (20000 rpm, 20 min) to reduce droplet size. To prevent the resulting droplets from merging, and to reduce the size further, a pre-warmed PVA solution (0.2% W/V, 50°C) was added, and homogenization was continued for 10 min. The emulsion was continuously stirred overnight for evaporation of the organic solvent and formation of nanoparticles. To remove the excess PVA and PEI, nanoparticles were washed twice with deionized water using centrifugation (14000 rpm, 30 min). The particles were used immediately or freeze dried (without adding any cryopreservatives) for later application.

3.2. Characterization of PLGA and PLGA/PEI particles

The average hydrodynamic diameter and zeta potential of the nanoparticles dispersed in phosphate buffered saline (PBS) (pH = 7.4) was measured by Dynamic Light Scattering (DLS) and laser Doppler electrophoresis, respectively (Zetasizer Nano-ZS; Malvern, UK).

The surface morphology of NPs was visualized by scanning electron microscopy (SEM) (XL-2930, Philips, Poland) at an accelerating voltage of 25 kV. For this reason, a drop of sample was instilled on an aluminum strip, air-dried and coated with gold before observation.

For investigating amide formation in PLGA/PEI NPs, Fourier transform infrared spectroscopy (FTIR) was used. The freeze-dried PLGA and PLGA/PEI were plated with KBr and analysis was conducted by Nicolet FT- IR Spectrometer (Magna-IR 550; Madison, WI) at 4 cm^{-1} resolution.

3.3. Preparation of polymer-based gene delivery vectors

A plasmid containing the gene encoding enhanced red fluorescent protein under control of cytomegalovirus promoter (CMV), (pLEX-jRED-puro; 11308 bp) was used in this study. With the aim of delivering the genetic material to hRPE cells, two different conformations of pDNA, PEI, and PLGA/PEI nanoparticles along with a vector composed of negatively charged PLGA instead of positively charged PLGA/PEI nanoparticles were constructed, and their transfection efficacy was compared (Fig. 1).