Nanoparticles for gene delivery to retinal pigment epithelial cells

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Purpose: To evaluate the safety and potential use of poly(lactic) acid (PLA) and poly(lactide-co-glycolide) (PLGA) nanoparticles (NPs) as vectors for gene transfer to RPE cells.

Methods: Experiments were conducted with primary bovine RPE cells and with the ARPE-19 human RPE cell line. Rhodamine loaded NPs were used to study factors influencing the internalization process by the various RPE cells: concentrations of NPs, duration of contact time, stage of cell culture and ambient temperature. The extent of NPs internalization was evaluated by fluorescence and phase microscopy. Potential NP toxicity was measured by the trypan blue exclusion dye test and the MTT method. Green fluorescent protein (GFP) plasmid or red nuclear fluorescent protein (RNFP) plasmid were sequestered in NPs. The ability of these “loaded” NPs to generate gene transfection and protein expression in RPE cells was assessed both in vivo and in vitro by fluorescence and confocal microscopy.

Results: The extent of NP internalization in cultured cells increases with their concentration reaching a plateau at 1 mg/ml and a contact time of up to 6 h. Temperature and culture stage did not influence the in vitro internalization process. No toxic effects on RPE cells could be detected when these were incubated with up to 4 mg/ml of NPs. In human and bovine RPE cells incubated with GFP loaded NPs, cytoplasmic green fluorescence was observed in 14±1.65% of the cultured cells. Incubation with RNFP loaded NPs yielded a nuclear red fluorescence in 18.9±1.6% of the cells. These percentage levels of expression initially detected after 48 h of incubation remained unchanged during the following 8 additional days in culture. No significant differences in the extent of cytoplasm or nuclear fluorescence expression were observed between bovine or human RPE cultured cells. In vivo, a preferential RNFP expression within the RPE cell layer was detected after intra vitreous injection of RNFP plasmid loaded NPs.

Conclusions: The ability of PLGA NPs to sequester plasmids, their nontoxic characteristics, and rapid internalization enables gene transfer and expression in RPE cells. These findings may be of potential use when designing future gene therapy strategies for ocular diseases of the posterior segment.

Retinal pigment epithelium (RPE) integrity and function are essential for neural retina homeostasis and play a major role in ocular diseases associated with senescence (as in age related macular degeneration) and in diseases associated with dystrophies of the photoreceptors. RPE cells therefore may serve as a potential targets for drug delivery and gene transfer aiming at arresting or reversing the processes leading to these diseases.

In spite of relatively reduced transfer efficiency, nonviral gene delivery systems bypass the hazards and immune reactions associated with viral vectors [1-3]. Poly(lactic) acid (PLA), poly(glycolic) acid (PGA), and their co-polymers (PLGA) are widely used by the biomedical industry. These polymers and their derivates are evolving as interesting matrices for encapsulated genetic material or drug [1,6]. It has been reported that PLGA NPs produce efficient transfer and sustained expression of an alkaline phosphatase gene [12,13].

In the present study, we assessed the in vitro kinetics of rhodamine loaded PLA NP uptake, the toxicity of PLA and PLGA NPs in bovine and human RPE cells, and the efficacy of PLGA NPs to mediate gene transfer in RPE cells in vitro and in vivo in the rat.
METHODS

Rhodamine loaded NPs preparation and characterization: Rhodamine-6G loaded PLA NPs were prepared by nanoprecipitation from DMSO, as described earlier [14,15]. Briefly, 64 mg of poly(D,L-lactide) (R-206, $M_w = 48$ kDa, and $M_w/M_n = 1.8$, Boehringer-Ingelheim, Ingelheim, Germany), 640 µg of a methacrylate random copolymer P(MMA-co-MA 12 ) that contained 12 mol% of MA (methacrylic acid; $M_n = 24$ kDa, $M_w/M_n = 1.2$) and 2.2 mg of Rhodamine-6G (Sigma, St. Louis, MO) were dissolved in 4 ml DMSO. Phosphate buffer (25 ml, 0.13 M, pH 7.4) were rapidly added to the polymer solution with no additional stirring. The suspension was first dialyzed against water (molecular weight cut-off of 6,000 to 8,000; Spectra/Por; Spectrum, Savannah, GA), precipitated at acidic pH to remove non-encapsulated Rhodamine and then resuspended in 32 ml of phosphate saline buffer (0.13 M, pH 7.4, NaCl 0.9%). The total Rhodamine (Rh) concentration (Rh sequestered in the nanoparticles and the free Rh in the suspension) determined by UV-visible spectrometry after dissolution of the lyophilized suspension in DMSO (max absorption wavelength in DMSO: 538 nm) was 59 µg/ml. The free Rh concentration in the supernatant was 3.3% of the total Rh with an Rh loading in the nanoparticles of around 2.5 wt%. Rh NPs were sterilized by filtration and stored at 4 °C for a maximum of 8 days before use [14].

The mean diameter of the nanoparticles measured by dynamic light scattering (Brookhaven, argon laser 488 nm, “contin” calculation method) was 140±20 nm. The zeta potential in PBS measured by doppler electrophoretic light scattering (Coulter Delsa 440, Coulter Corp., Hialeah, FL) was -60 mV.

Prior to their use, the PLA NPs preparation is centrifuged at 3000 rpm for 3 min, the supernatant collected and used for the experiments.

Plasmid DNA: A plasmid encoding for green fluorescent protein (GFP), pIRES-ECGFP (Clontech, Palo Alto, CA) and a plasmid encoding for red fluorescent protein fused with three copies of the nuclear localization signal NLS (NRFP; pDsRed2-Nuc; Clontech) were studied. The plasmids were amplified in Escherichia coli host strain DH5α, extracted by alkaline lysis technique, and purified using maxiprep column isolation kit (Qiagen, Hilden, Germany).

Plasmid loaded NPs preparation and characterization: A double emulsion system and the solvent evaporation technique were used to incorporate the pDNA in PLGA [13]. Briefly, 200 µl of Tris-EDTA buffer (TE; 0.1 mM Tris and 10 mM ETDA) containing 500-1000 µg of plasmid were emulsified in 3% PLGA/chloroform solution (3 ml) using a homogenizer (Omni TH, Omni international, Warrenton, VA) at 30000 rpm for 1.5 min. The resulting primary emulsion was added dropwise to a 2% PVA solution (in 25 ml TE) and homogenized for 4 min (double emulsion). Following overnight evaporation at 4 °C the formed particles were collected by ultracentrifugation at 80000 g, washed three times with sterile double distilled water, resuspended, and lyophilized. The amount of plasmid DNA entrapped in the NPs (7.36 µg DNA/1 mg NPs for GFP and 4.5 µg DNA/1 mg NPs for Red-Nuc) was determined by dissolving the NPs in chloroform and extraction of the DNA by repetitive addition of TE buffer. Structural integrity of the extracted plasmids was analyzed by gel electrophoresis before and after enzymatic digestion.

Plasmid loaded NPs size was 643±74 nm as measured by laser light scattering (Coulter N4 submicron particle size analyzer, Luton, UK). Zeta potential was -7.4±3.3 mV as measured by a Zetamaster equipment (ZEM, Malvern instruments, Orsay, France).

Post-labeling of pDNA-NPs with the DNA fluorescence probe Et-Br showed a fluorescence signal in over 80% of the NPs.

Figure 1. Fluorescence microphotographs of bovine RPE cells incubated with fluorescent nanoparticles. Fluorescence microphotographs of bovine RPE cells incubated with fluorescent nanoparticles for 6 h, showing that intracellular content of nanoparticles depend on their concentration in the medium. RPE cells incubated with 0.1 mg/ml (A) and 4 mg/ml (B) of NP-Rh for 6 h. Ingested nanoparticles appear as red fluorescent dots. Bars represent 20 µm.
Cell isolation and culture: Bovine RPE: Bovine RPE cells are isolated from fresh enucleated calf eyes obtained from a local slaughterhouse. Isolation and primary culture of RPE cells were performed as suggested earlier and with minor adaptations [16]. The anterior segment, vitreous and retina are removed and the remaining eyecup washed twice in PBS. After washing, trypsin/EDTA 0.2% is added, “bathing” the RPE cell layer for a 30 min digestion cycle. Loosened RPE cells are aspirated and transferred to centrifuge tubes containing DMEM supplemented with 10% fetal calf serum. This cellular suspension centrifuged at 1500 rpm for 5 min, was resuspended in DMEM/10% FCS (supplemented with L-Glutamine, penicillin, and streptomycin), plated in 6 well tissue culture plates and incubated in 95% O₂ and 5% CO₂ atmosphere at 37 °C. When reaching near confluence (2 to 3 days later), cultured cells are replated twice and used for the various tests. During this period, cultured RPE cells preserved their morphology and pigment granules. Cytokeratin staining, before experimentation, demonstrated the presence of at least 95% homogeneous population of RPE (cytokeratin positive) cells in all cultures.

Human RPE: The human RPE cell line ARPE-19 (American Type Culture Collection, Rockville, MD) was maintained in DMEM/F12 with 15 mM HEPES buffer, 2 mM L-glutamine, 5% calf serum and 1% insulin-transferrin-sodium selenite (ITS) and used at the third to fourth passages for all experiments. The ARPE cell line was maintained in a 37°C, 5% CO₂ incubator, and the medium was changed every 2 days.

Figure 2. Kinetics of nanoparticle internalization by bovine RPE cells. Phase and fluorescence microscopy of RPE cells incubated with 1 mg/ml NP-Rh. Removing the added nanoparticles (1.0 mg/ml) after 1 h of incubation and extending the culture for 72 h shows that each RPE cell in culture harbors a few NPs in its cytoplasm. The number of internalized NPs per cell increases rapidly when the contact time between cultured RPE cells and NPs is increased up to 6 h. Extended NP incubation times do not further increase internalization. A: Incubated 1 h. B: Incubated 3 h. C: Incubated 6 h. Bars represent 20 μm.
and 10% FCS [17]. Aliquots of the same batch were used throughout.

In vitro internalization of NP: All experiments were carried out in triplicate. 15000 cells in 0.5 ml of culture medium were seeded in 4 well Labtek (Nunc, Naperville, IL). The extent of NP internalization within the RPE cells was assessed by phase and fluorescence microscopy (Aristoplan, Leica, Heidelberg, Germany). The average number of internalized NPs per cell was obtained by direct counting of the particles under a fluorescent microscope after 4% paraformaldehyde fixation of the cells in the Labtek plates (Nunc).

Free rhodamine (2 µg/ml), blank PLA NPs (1 mg/ml), and medium were used as controls in these experiments.

Using rhodamine loaded NPs, the influence of several parameters on the extent of NPs internalization by bovine and human RPE cells were studied:

1. Concentration of NPs: Cells were incubated for 6 h with 0.01, 0.1, 1, and 4 mg/ml of NPs in 0.5 ml of medium. Remaining NPs were removed by three washing and new medium was added and the cultures were examined 72 h later.

2. Duration of NP contact with RPE cells: After 48 h of culture, cells were incubated with 1 mg/ml of NPs for 1, 3, 6, 24 and 48 h. The remaining NPs were washed out. The cultures were examined 72 h later.

3. Culture stage of the RPE cells: 1, 6, 24, 48 and 72 h after cell seeding, RPE cells were incubated for 6 h with 1 mg/ml NPs. The medium was then changed and the extent of NP internalization by the different cultures examined 24 h later.

4. Temperature: To determine the influence of temperature on NP internalization, RPE cells cultured for 72 h were placed at 4 °C for 4 h prior to the addition of 1 mg/ml NPs. After addition of the NPs, these cultures were further incubated for 6 h at 4 °C. After changing the medium, the cells were reincubated at 37 °C for 24 h and examined.

Effect of NPs on RPE cell number and viability: Potential toxicity of PLA and PLGA blank NPs was evaluated on bovine and human RPE cells. The effect of 0.01, 0.1, 1, and 4 mg/ml NPs on cell number and viability was assessed by two different methods: The trypan blue exclusion dye test and the MTT test. RPE cells cultured for 72 h were incubated with NPs for 24 h, washed, the medium replaced and examined three days later.

All experiments were performed in triplicate. For trypan blue exclusion dye test, 36000 cells in 1.5 ml were seeded into the 24 wells plates and for the MTT test, 1500 cells in 0.2 ml of culture medium seeded into each of the 96 wells of multi dish culture plates [18].

The MTT test was carried out as previously described [19]. Briefly, RPE cell cultures were rinsed three times with PBS, and 100 µl of MTT (Sigma, 1 mg/ml MTT in PBS containing 1 g/l glucose) was added to each well. After 1 h incubation at 37 °C, the MTT solution is removed. The resulting insoluble formazan crystals from this reaction are dissolved in 100 µl of propanolol and absorbance spectroscopy measured at 540 nm (control 630 nm) using an adapted 96 well plate spectrophotometer. The color intensity allows for the evaluation of the number of viable cells using a standard curve for each experiment [19].

In vitro transfection: Bovine and human RPE cells were seeded in 4 well Labtek culture plates (Nunc), and 3 days later (when reaching near confluence) NPs encapsulating either a GFP or a RNFP plasmid were added to the cultures at concentrations of 0.2, 2.0 and 4 mg/ml. Based on the observations regarding the kinetics of NPs internalization, “transfection” efficiency of the encapsulated plasmids was evaluated after a contact time of 6 h. The culture medium was removed after this period of contact, the cells washed thoroughly and further incubated with fresh medium. These RPE cell cultures were examined daily under an inverted fluorescent microscope (Leica DM IRB, Wetzlar, Germany), and at days 4 and 10 by confocal microscopy (Zeiss LSM510, Jena, Germany).

As control, naked, non-encapsulated plasmid was used on cell cultures. The concentrations of naked plasmid correspond to the concentration of plasmid encapsulated in 4 mg/ml NPs. Therefore, cells were incubated for 6 h with 29.44 µg/ml GFP plasmid DNA or with 18 µg/ml RNFP plasmid DNA. Cells were then washed, incubated with free medium, and examined as described above. Untreated cells were used as control as well. Experiments were performed in duplicate and reproduced twice.

The cytoplasm (GFP) or nuclear (RNFP) plasmid expression was recorded daily for 10 days in culture. The number of cultured RPE cells per field expressing cytoplasmic green fluorescence (for GFP NPs) or nuclear red fluorescence (RNFP NPs) and the total number of cells per 10 fields (magnification 25x) were calculated. Gene transfer efficiency was expressed as the average percentage of cells exhibiting a green cytoplasm or red nuclear fluorescence in each culture type.

Data analysis: Results were expressed as means±SEM (standard error of the mean). Statistical differences between groups were assessed by the Mann-Whitney nonparametric
test. p<0.01 was considered to be significant.

**In vivo transfection:** Male Lewis rats, 6-7 weeks old and weighing 150-200 g (IFFA CREDO, Lyon, France), were used. Experiments were conducted in accordance with the ARVO Statement for the Use of Animals in Ophthalmic and Vision Research. Rats were held in the vivarium for 1 week before inclusion in the study.

For the experimentation, rats were anesthetized by intraperitoneal pentobarbital injection (40 mg/kg) and the right eyes dilated using Tropicamide 0.5% eye drops. Intravitreous injections of 5 µl (4 mg/ml) RNFP loaded NPs (group I, n = 3) or blank NPs from the same batch (group II, n = 3) were performed. Additional control eyes received 5 µl of “naked” RNFP (18 µg/ml) plasmid (group III, n = 3) or 5 µl of balanced saline (BSS, Alcon, Fort Worth, TX; group IV, n = 3). The injections were performed using a 30 gauge needle inserted at 11 o’clock, 2 mm posterior to the limbus.

Eyes were examined by indirect ophthalmoscope after the intra vitreous injection. If lens trauma or vitreous hemorrhage occurred, these eyes were excluded from the study and replaced in order to have a minimum of two eyes (two animals) for each treatment group and each examination time point. On day 4, 7, and 14 two rats from each group were sacrificed, the right eyes enucleated, snap frozen in OCT and processed for fluorescence microscopy.

**RESULTS**

*Internalization of NPs:* No differences were observed between bovine primary cultures and human RPE cells lines regarding their internalization abilities. Therefore, the results obtained with bovine RPE cells are represented unless otherwise specifically stated.

1. Effect of NP concentration: Examination of the cultured RPE cells with a fluorescent microscope shows that the number of ingested particles per RPE cell and the relative number of ingesting cells within the culture increases in parallel with the initial added NP concentration reaching a plateau at 1.0 mg/ml. At 0.01 mg/ml, the mean number of NPs/cell was 9.77±3.31, at 0.1 mg/ml, 22.17±8.5; at 1 mg/ml, 218±59 and at 4 mg/ml, 213.17±86 (n=6). There was no significant difference between the number of NPs/cell between 1 mg/ml and 4 mg/ml (p=0.94) while the difference was significant between 0.01 and 0.1 (p=0.009) and 0.1 and 1 mg/ml (p=0.002; Figure 1).

2. Effect of contact time duration between NPs and RPE cells: Kinetics of NPs internalization by bovine RPE cells shows that the process is rapid. Removing the added NPs (1.0 mg/ml) after one hour of incubation and extending the culture for 72 h shows that each RPE cell in culture harbors a few NPs in its cytoplasm (average 19±11 NPs/cell; Figure 2A). The number of internalized NPs per cell increases rapidly when the contact time between cultured RPE cells and NPs is increased to 3 h (average 52±9 NPs/cell; Figure 2B), or to 6 h (average 224±98 NPs/cell; Figure 2C). Extending NPs incubation time to 24 or 48 h does not induce a further increase in the average number of ingested NPs per RPE cell.

3. Culture stage of the RPE cells: Performing these experiments when the contact between RPE cells and NPs is initiated immediately after cell plating (when the RPE cells are still in suspension) or at various intervals thereafter, including the stage of near cell culture confluence, did not have a significant influence on NP internalization pattern (data not shown).

![Figure 4](http://www.molvis.org/molvis/v11/a14)

**Figure 4.** Gene expression in cultured RPE cells incubated with nanoparticles loaded with GFP plasmid. Gene expression in cultured RPE cells incubated with 2 mg/ml of nanoparticles loaded with GFP plasmid. Cultured bovine RPE (A) and human RPE (B) cells incubated with 2 mg/ml of nanoparticles loaded with GFP plasmid. Cytoplasmic green fluorescence (GFP expression) is detected in some cells on day 4 of culture. Bovine RPE (C) on day 4 incubated with 29.44 µg/ml of naked GFP plasmid. No cytoplasm fluorescence is observed. Bars represent 20 µm.
4. Influence of temperature on internalization: The NP cell internalization pattern was not influenced by incubation of the cultures at either 37 °C or 4 °C, indicating that NP internalization by RPE cell is not associated with specific metabolic activity of the cultured cells.

Effect of NPs on RPE cell number and viability: Addition of PLA NPs at a concentration up to 4 mg/ml and a contact time as long as 48 h did not induce any cell toxicity nor there was any detectable interference with the metabolic and proliferation abilities of the cultured RPE cells (Figure 3).

Figure 5. Bovine RPE cells incubated with nanoparticles loaded with RNFP plasmid. Detectable nuclear red fluorescence was observed in bovine RPE cells incubated with 4 mg/ml of NPs encapsulating the RNFP plasmid. This fluorescence was observed after 48 h of incubation and lasted up to 10 days. A: Phase and fluorescence microscopy of bovine RPE cells on day 6 of culture. B-D: Confocal microscopy of the same cultures: red fluorescence (B), phase (C) and combined phase and red fluorescence (D). Red fluorescence (expression of RNFP) is strictly confined to cell nuclei. Bars represent 20 μm.
Similar results were observed when PLGA NPs were used. The number of viable cells in the control culture wells (without NPs) and in cultures exposed to the different NP concentrations throughout the periods of observation remained similar with slight differences not reaching statistical significance ($p<0.05$).

Human RPE cell line cultures demonstrated a similar pattern of NP internalization behavior as the bovine RPE cells (not shown).

**Gene expression in cultured RPE cells:** No green fluorescence was observed in the cytoplasm of any of the RPE cells during the first day after ingestion of the NPs encapsulating the GFP plasmid. After 48 h, an initial cytoplasm expression of green fluorescence was observed in 14.21±1.65% of the cells (range 10 to 35%). No significant change in the proportion of cells expressing GFP was detected during the extended period of up to 10 days observation of these cultures. The mean and percent ranges of bovine RPE cells (Figure 4A) were similar to those observed in human RPE cultures (Figure 4B). Isolated fluorescent RPE cells (<1%) were detected in cultures exposed to naked plasmid (29.44 µg/ml GFP plasmid or 18 µg/ml RNFP plasmid). These concentrations correspond to the amount of plasmid encapsulated in the loaded NPs (Figure 4C). No cytoplasmic green or nuclear red fluorescence was observed in RPE cell cultures incubated with blank NPs (not shown).

Detectable nuclear red fluorescence was observed only when using the 4 mg/ml of NPs encapsulating the RNFP plasmid. At this concentration, an average of 18.86 ±1.59% RPE cells (range 15 to 35%) showed a homogeneous red nuclear fluorescence. This fluorescence was also observed only after 48 h of incubation and lasting up to 10 days later. No significant change in the proportion of cells expressing RNFP was detected during the observation period. As with the GFP encapsulating NPs, a large proportion of the RPE cells did not demonstrate any fluorescence despite the extended incubation period of 10 days (Figure 5).

**Gene expression in vivo:** Gene expression of RNFP (red nuclear fluorescence) was observed within the RPE cells as soon as four days after intracocular injection. On day 7, some cells expressing RNFP were observed in the inner retinal layers, but most of the expression was localized within the RPE layer. Protein expression remained apparent in RPE cells on day 14 (Figure 6). The fluorescence was clearly located within RPE nuclei. Controls injected either with blank NPs or with naked plasmid coding for RNFP showed no fluorescence at
any time point (not shown). On histology, no apparent structural damage or toxic effects were induced by the NPs injection.

**DISCUSSION**

Nonviral gene delivery to RPE cells has been studied using lipoplexes (such as Lipofectin and Lipofectamine) and polyplexes (e.g., polyamidoamine starburst dendrimers, polyethylenimines). These systems yield up to 20% transfection efficiency [20-22]. But, lipoplex toxicity is a limiting factor for their potential therapeutic application [23]. In our present study, 10% to 35% of cells expressed the transfected gene, but no cell toxicity was observed even when high PLGA NPs concentrations (up to 4 mg/ml) were added. Furthermore, polymeric NPs can be used in the presence of serum allowing for their use with in vivo systems as shown in earlier studies [24]. Moreover, small size PLGA NPs injected into the vitreous of rat eyes follow a trans-retinal pathway and undergo a rapid internalization into the RPE cells [14,25].

PLA and PLGA have been approved for human use by the US Food and Drug Administration. They are biodegradable, biocompatible, and are among the most extensively investigated polymers for drug delivery purposes [26,27]. By changing the copolymer composition and molecular weight, the release of encapsulated drugs or DNA fragments from the PLA or PLGA matrixes can be altered from days to months [28]. Higher encapsulation efficiency and longer intracellular sustained release rates might preclude the need for repeated in vivo administration, allowing for a potential long standing therapeutic effect.

Different cell lines and types may differ in their ability to undergo transfection [23]. In our present study, we used RPE cells from differing origins: Human cell line and bovine primary cultures [29-31]. Internalization kinetics of NPs loaded with two different plasmids (GFP with expression of protein within the cell cytoplasm and NRFP with expression in the cell nucleus) was similar for all types of RPE cells studied. NRFP may be of interest for in vivo transfection of RPE cells, avoiding interference with the often observed cytoplasm auto fluorescence of various intraocular tissues and RPE cell layer in particular. It is of interest that RPE cells from different origins demonstrated similar internalization and expression profiles. Earlier works have as well shown that ARPE-19 cells can internalize nanoparticles through nonsaturable mechanisms, with the uptake increasing with decreasing size [32].

The efficiency of gene transfer using polyplex NPs relies on intracellular trafficking. It was shown that following their uptake, this type of NPs escape the early endo-lysosomal formation and enter the cytosolic compartment by a mechanism of surface charge reversal [6,33]. Thus, the potential therapeutic efficacy of these NPs is enhanced by the shortened contact time with lysosomal enzymes [6] and by their ability to protect the encapsulated plasmids from degradation by lysosomal nucleases [5,34].

Polymeric NPs can be used to deliver to RPE cells other types of nucleic acid fragments, such as oligonucleotides. It was recently showed in the ARPE-19 cell line that PLGA nanoparticles enhance cellular delivery of an encapsulated VEGF antisense oligonucleotide, and inhibit VEGF secretion and mRNA expression, while no effect was observed with the free oligonucleotides [35].

As we had observed earlier [14], NPs injected in the vitreous localize within the RPE cells within the first 24 h. From our preliminary in vivo observations, once the NPs encapsulating the plasmid are within the RPE cells, expression of the proteins is detected initially as soon as the fourth day after injection remaining detectable for the following three weeks. Ongoing experiments in our laboratory aim to determine whether one single intra vitreous injection or repeated NPs injections are needed in order to achieve a long standing and sustained gene expression within the RPE cells. Interestingly, in vivo expression of plasmid protein seems to be higher than the in vitro expression. This could be related to the in vitro division of cells leading to a “dilution” of the released plasmid, while it could accumulate in nondividing RPE cells in vivo. Another explanation could be related to an enhanced release of plasmid in RPE cells in vivo when intense specific phagocytosis is occurring.

Our present study demonstrates that PLGA polyplex NPs are nontoxic and can be used to achieve a gene transfer into RPE cells in vitro and in vivo. Further in vivo experiments are now carried out in order to evaluate the in vivo potential value of these early observations.

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