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Synergistic effects between natural histone mixtures and polyethylenimine in non-viral gene delivery in vitro

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ABSTRACT

Nanoparticles made of plasmid DNA (pDNA) and cationic polymers are promising strategies for non-viral gene delivery. However, many cationic polymers are toxic to cells when used in higher concentrations. Positively charged proteins, such as histones, are biodegradable and a good alternative, especially for potential in vivo applications. It has previously been shown that histones are able to complex DNA and mediate transfection of cells. To investigate possible synergistic effects between the different histone types and to avoid the use of recombinant proteins, we analysed whether natural histone mixtures would be functional as gene carriers. Core and linker histones from calf thymus and from chicken erythrocytes were used to transfect different cell lines. The protein mixtures efficiently complexed the pDNA, and the resulting particles entered the cells. However, only marginal expression of the gene encoded by the pDNA was observed. Transfection rates increased drastically when minimal amounts of the basic polymer polyethylenimine (PEI) were added to the particles. Neither PEI nor histones alone mediated any transfection under the conditions where a combination of both worked efficiently, and the combined particles were well tolerated by the cells. These results demonstrate that histone mixtures from natural sources in combination with minimal amounts of PEI can be used as gene carriers. This might have consequences for the development of novel gene delivery strategies, such as DNA vaccines, with minimal side-effects.

1. Introduction

Non-viral gene delivery to eukaryotic cells is hampered by multiple cellular barriers, such as the cell membrane, intracellular crowding, nuclease degradation and the nuclear envelope (Lechardeur and Lukacs, 2006). A variety of different technologies were developed to overcome these barriers in order to make non-viral gene delivery an efficient process. An established strategy is the complexation of DNA with basic polymers or basic proteins. Thereby the negative charge of the DNA molecule is neutralized, and particles are generated which are taken up by the cell via endocytosis. Several cationic polymers are used for gene delivery. One of the most effective ones is polyethylenimine (PEI) (Boussif et al., 1995; Pollard et al., 1998; Garzon et al., 2005). PEI efficiently complexes DNA and protects it against endolysosomal degradation (Godbey et al., 1999; Oh et al., 2002). In addition, it releases polymer–DNA complexes from endosomes, which is an advantage over other polymers. However, for optimal transfection rates high amounts of PEI have to be used, which causes problems due to the intrinsic cytotoxicity of the compound (Boussif et al., 1995; Kawakami et al., 2006). In light of potential in vivo applications, this is of concern (Chollet et al., 2002). As an alternative to cationic polymers, positively charged proteins were also shown to deliver foreign DNA. These are biodegradable and toxic effects are less likely. Among the proteins investigated in transfection studies are histones (Kaouass et al., 2006), which are evolutionary highly conserved, small, basic and DNA binding proteins. It was shown previously that a recombinant human H1 histone fragment is able to efficiently transfect cells (Fritz et al., 1996; Puebla et al., 2004). Also H1 purified from calf thymus nuclei was demonstrated to be a (calcium-dependent) gene carrier (Haberland et al., 2000; Zaitsev et al., 2002). Other studies used recombinant H2A and H2B to deliver DNA to different cell lines (Balicki et al., 2002; Wagstaff et al., 2007). Hence, experiments using single histone proteins showed that these are capable to transfect cells under various conditions. However, it is not known to date whether synergistic effects are seen when different histones are combined, e.g. in natural mixtures, which consist of all core histones and the linker histone H1. Therefore, we analysed different

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natural histone for their ability to deliver foreign DNA to cultured cells.

2. Materials and methods

2.1. Materials and cells

Calf thymus and chicken erythrocyte histones were purchased from Roche Diagnostic and Meridian Life Science (USA), respectively. Linear 22 kDa polyethylenimine was obtained from Fermentas. Vero V76 (vervet monkey, kindly provided by Prof. Liebert, University of Leipzig) cells were grown in DMEM supplemented with 5% FCS and 1% antibiotics. BHK-21 (baby hamster kidney, Sigma, Deisenhofen, Germany) cells, clone 13, were grown in GMEM, supplemented with 10% FCS, 5% Tryptose Phosphate Broth (Sigma, Taufkirchen, Germany) and 1% antibiotics. Media und supplements were from Invitrogen (Karlsruhe, Germany).

2.2. Preparation of plasmid DNA

Transfection experiments were performed using a pVAX1 plasmid (Invitrogen) encoding the enhanced green fluorescent protein (EGFP) gene driven by the human cytomegalovirus (CMV) promoter. This vector (pVAX/EGFP) was propagated in *Escherichia coli* TOP10 cells (Invitrogen). Plasmid DNA was purified by endotoxin-free GigaPrep Kit from Sigma–Aldrich according to the manufacturer's instructions. DNA concentration and purity were determined by UV spectroscopy.

2.3. Formation of the different pDNA complexes

Plasmid DNA (6 μg), diluted in 250 μl of a 150 mM sodium chloride (NaCl) solution, was complexed by the amounts of histone mixtures indicated (Figs. 1–6) to obtain pDNA–histone complexes. The solution was vortexed and incubated for 30 min at room temperature.

For particles of pDNA, histones and PEI (pDNA/H–P), pDNA (6 μg) and the desired amount of corresponding histones were mixed in 250 μl 150 mM NaCl and vortexed. After 20 min, PEI at the defined molar PEI-nitrogen and pDNA-phosphate ratios (N/P) of 2 or 3 was added, mixed, and the resulting solution was incubated for further 10 min. We also tested particles which were assembled by complexing pDNA first with PEI and subsequently with histones (pDNA/P–H). Since comparable transfection results were obtained with both methods (data not shown), particles formed by mixing pDNA and histones first were used in the following experiments. pDNA–PEI particles were composed of 6 μg pDNA diluted in 250 μl 150 mM NaCl and PEI at the N/P ratios indicated. The mixtures were incubated for 10 min at room temperature before use. Alternatively, all particles were also assembled in 5% glucose. For reasons which remain to be determined, these performed far less effectively in the transfection experiments compared to the ones assembled in

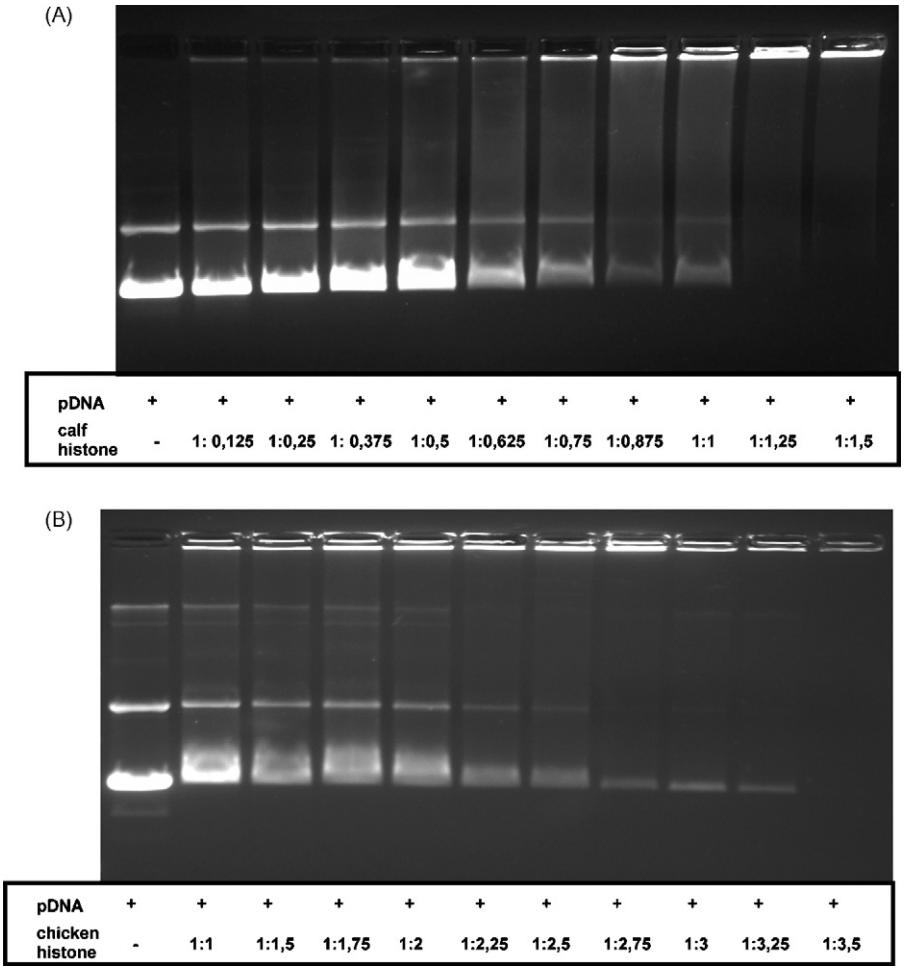

Fig. 1. Plasmid DNA retardation assay: increasing amounts of natural histone extracts from calf thymus (A) and chicken erythrocytes (B) were incubated with pDNA and subjected to electrophoresis on a 0.75% agarose gel. As control, naked DNA was used (lane 1).

150 mM NaCl (data not shown). Hence, particles in glucose were not further studied in this work.

2.4. Gel retardation assay

Two micrograms of plasmid DNA were incubated with increasing amounts of histones (as indicated in Figs. 1 and 4), calf or chicken type, and/or PEI (N/P 2 or 3) in 50 μl of a 150 mM NaCl solution. After an incubation of 30 min at room temperature the resulting complexes were loaded into wells of a 0.75% agarose gel containing ethidium bromide and electrophoresed at 60 V for 3 h. Subsequently, pDNA bands were visualized under UV light and photographed.

2.5. In vitro transfection experiments and flow cytometry

Twenty-four hours before transfection Vero V76 and BHK-21 cells were seeded into 6-well plates at a density of 3×10^5 and 2.5×10^5 cells/well, respectively. Immediately before start of transfection experiments, cells were washed with phosphate buffered saline (PBS). Two milliliters serum- and antibiotics-free transfection medium containing the corresponding pDNA particles (6 μg pDNA/well) were added. After 4 h at 37 °C transfection medium was replaced by culture medium without antibiotics but with FCS. EGFP expression was monitored 48 h later by flow cytometry. Cells were rinsed with PBS, trypsinized, collected by centrifugation and finally resuspended in PBS containing 1% FCS. Transfection efficiency is expressed as the percentage of EGFP expressing cells at an excitation wavelength of 488 nm using a BeckmanCoulter FC500 cytometer (BeckmanCoulter, USA).

2.6. In vitro DNA tracking

Plasmid DNA was labelled with fluorescein using the *Label* IT® Fluorescein Labeling Kit from Mirus Bio LLC (USA). pDNA tracking was analysed by flow cytometry as described above and by fluorescence microscopy. For the latter, Vero V76 (6×10^4 cells/well) cells were seeded on cover slips in a 24-well plate. After 1 day, cells were washed with PBS and medium was replaced by transfection medium. Fluorescein-labelled pDNA (1.4 μg) was again complexed by histones and PEI adjusted to the corresponding pDNA concentration and incubated with cells for 2 days. Cells were rinsed twice with sodium citrate buffer (25.5 mM citric acid, 24.5 mM sodium citrate, 280 mM sucrose, 0.01 mM deferoxamine mesylate, pH 4.6) to remove cell surface-bound particles (Ghosh et al., 1994; Grosse et al., 2007), followed by two washing steps with PBS. Cells were fixed with 0.5 ml 3.7% formaldehyde, washed with PBS and subsequently stained with 4′-6-diamidino-2-phenylindole (DAPI) for 15 min. Finally, the cover slips were rinsed five times with PBS and mounted on glass slides. Samples were analysed under a DMI4000 fluorescence microscope (LEICA, Germany) at 1000× magnification.

2.7. Cell viability assay

Both Vero V76 and BHK-21 cells were seeded in 6-well plates and transfected with pDNA particles as described above. After 2 days viable cells were counted by using trypan blue for excluding dead cells.

2.8. Dynamic light scattering experiments to measurement size of pDNA-bound particles

The average particle size was determined using the Zetasizer ZS (Malvern, Worcestershire, UK). The particle size and polydispersity index were measured in triplicate in an undiluted dispersion of particles at 25 °C.

3. Results

3.1. Evaluation of histone extracts for pDNA complexation

First, we tested the DNA binding capacity of natural histone mixtures in a gel retardation assay. Plasmid DNA (pDNA) was incubated with increasing amounts of histones and analysed on an agarose gel. Fig. 1A shows pDNA complexation by calf thymus histones. At a pDNA–histone ratio (w/w) of 1:0.5 a slight DNA shift was observed which became stronger with increasing amount of histones. At a ratio of 1:1.25, DNA was completely complexed by histones and remained in the slot. In comparison, chicken erythrocyte histones required a higher concentration of proteins for complete packing of plasmid DNA. This was finally achieved at a pDNA–histone ratio of 1:3.5 (Fig. 1B). These results demonstrate that both histone extracts are capable to complex plasmid DNA in a concentration-dependent manner.

3.2. In vitro transfection efficiency of pDNA–histone complexes

Next, histones were analysed for their capability to transfect cells. A DNA plasmid containing the EGFP gene under the control of a CMV promoter was generated and protein expression after transfection was measured by flow cytometry. Two different fibroblastic cell lines, Vero V76 and BHK-21, were transfected with pDNA–histone complexes at varying weight-ratios. Two days later, cells were analysed for EGFP expression. Complexation of pDNA with histones alone, calf or chicken type, was very ineffective to detectably transfect Vero V76 cells (Fig. 2A and B). EGFP expression was not significantly increased compared to negative controls or cells incubated only with the naked pDNA. Similar results were measured in BHK-21 cells (Fig. 2C and D). Here, at extremely high pDNA–histone ratios of 1:128 a transfection efficiency of approximately 10% was observed. However, these pDNA–histone ratios were strongly toxic on both cell lines (data not shown), hence we did not investigate this finding further. With further increase of histones the complexes precipitated and thus were useless for transfection experiments.

3.3. In vitro transfection efficiency of particles containing pDNA, histones and PEI

As pDNA–histone complexes did not transfect cells to reasonable levels, we tried to optimise these particles. The cationic polymer polyethylenimine (PEI) was previously shown to enhance transfection effects of other pDNA–protein complexes when applied at high concentrations (Rhaese et al., 2003; Shen et al., 2009). However, high transfection efficiency mediated by high amounts PEI correlates with cytotoxicity (Boussif et al., 1995; Kawakami et al., 2006). To avoid cytotoxicity of PEI, we applied the polymer at very low, non-toxic concentrations (N/P ratios 2 and 3) to the preformed histone–DNA particles and we refer to these complexes as pDNA/H–P particles. Similar to pDNA–histone complexes, the pDNA/H–P complexes and pDNA–PEI particles (with PEI at N/P 2 and 3) completely complexed the pDNA in a gel retardation assay (Figs. 1 and 3).

pDNA/H–P particles were administered to the two fibroblastic cell lines, Vero V76 and BHK-21. Transfection experiments were done as described and EGFP expression was measured by flow cytometry 2 days post-transfection. Vero V76 cells transfected with naked pDNA or pDNA–PEI particles (N/P ratio 3) showed no transfection effect (Fig. 4A and B), similar to the negative control and in accordance with previous studies (Honoré et al., 2005). In contrast,

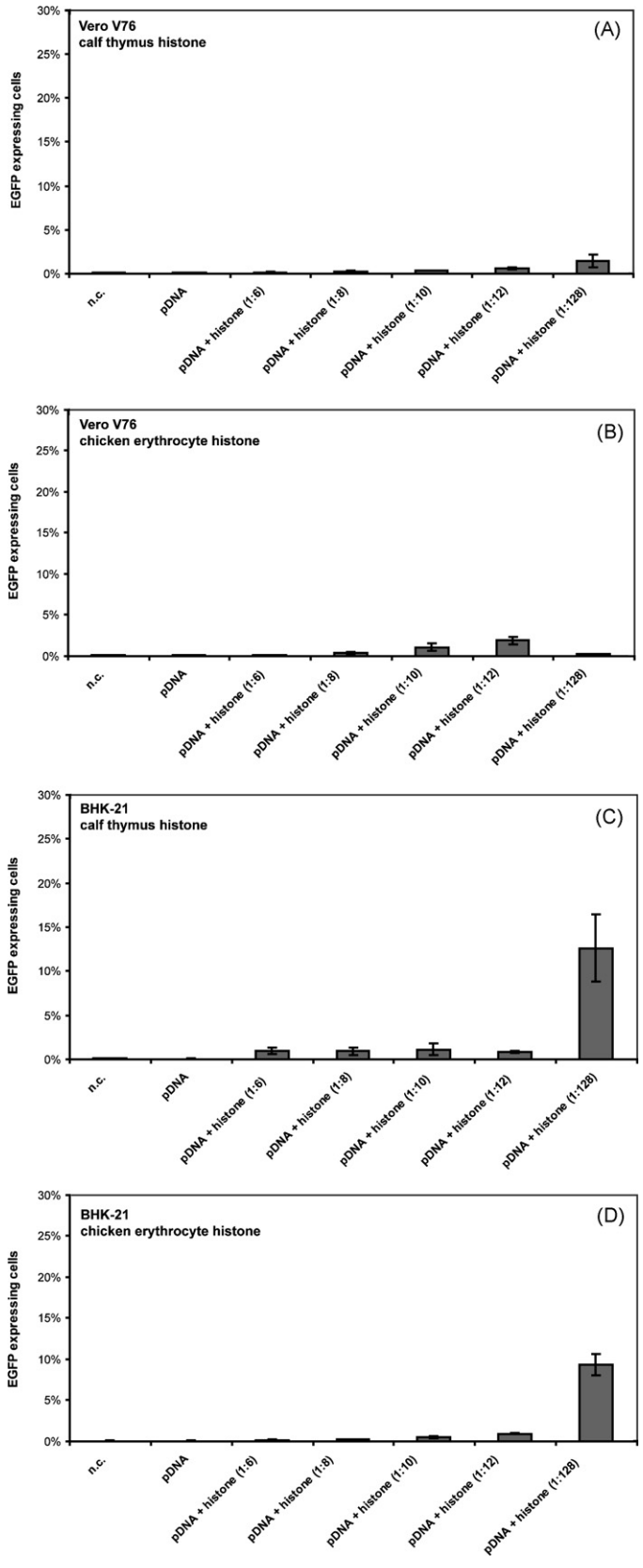

Fig. 2. Transfection efficiencies of pDNA–histone complexes in Vero V76 (A and B) and BHK-21 (C and D) cells, 48 h post-transfection. EGFP expression was measured by flow cytometry. pDNA was assembled in particles with histones from either calf thymus (A and C) or chicken erythrocytes (B and D). Nontransfected cells were used as negative controls (n.c.). Mean values of 3–5 experiments. Error bars represent the standard deviation (SD).

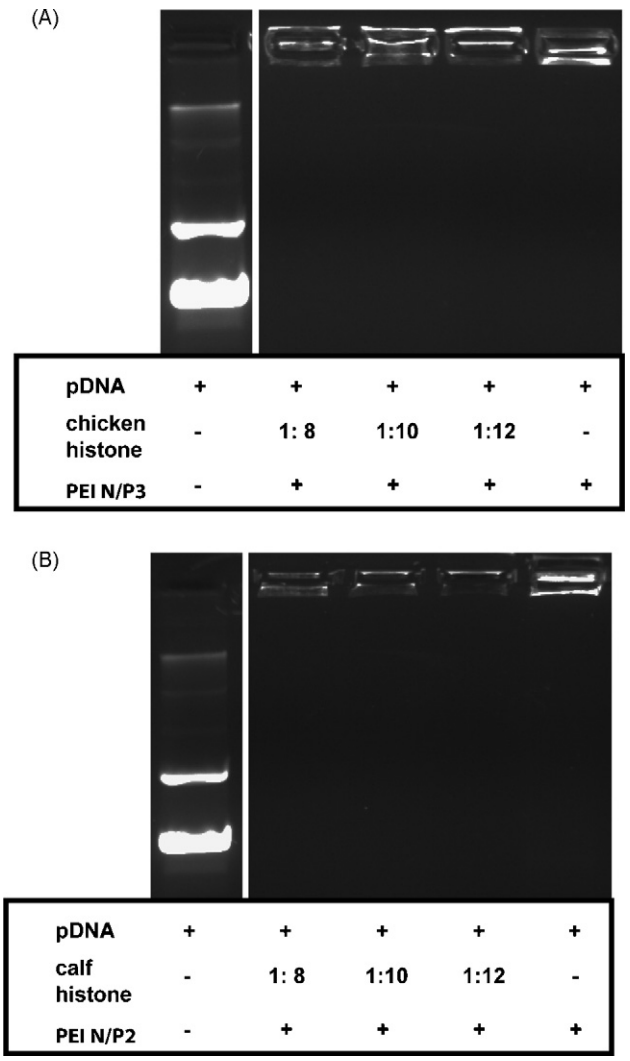

Fig. 3. Plasmid DNA retardation assay with pDNA/H–P particles and pDNA–PEI complexes. (A) pDNA was complexed with chicken histones in varying concentrations and/or PEI at a N/P ratio of 3. (B) pDNA/H–P composed of calf histones and/or PEI (N/P 2). Complexes were subjected to electrophoresis on a 0.75% agarose gel. As control, naked DNA was used (lane 1).

pDNA/H–P particles containing increasing amounts of chicken histones led to a significant EGFP expression up to approximately 20% (at a pDNA–histone ratio of 1:12) of transfected cells (Fig. 4A). The same particles containing calf histones were less efficient (Fig. 4B). In Fig. 4C, transfection efficiencies of pDNA/H–P particles containing PEI at a N/P ratio of 2 are shown in Vero V76 cells. These particles also led to an increased EGFP expression compared to naked pDNA or pDNA–PEI particles (N/P 2). However, these particles were less efficient than particles containing PEI at an N/P ratio of 3.

Similar results were obtained with BHK-21 cells (Fig. 5). Here, pDNA was complexed with calf histones followed by addition of PEI at a N/P ratio of 2. Transfections with these particles increased EGFP expression beyond 20% whereas particles consisting only of pDNA and PEI at N/P 2 (pDNA–PEI) failed to transfect at detectable levels (Fig. 5A). In these cells, usage of chicken histones in complex assembly resulted in lower transfection effects (Fig. 5B).

In contrast to Vero V76 cells, usage of PEI alone at an N/P ratio of 3 was enough to significantly transfect BHK-21 cells, and addition of histones did not further increase the effect (Fig. 5C).

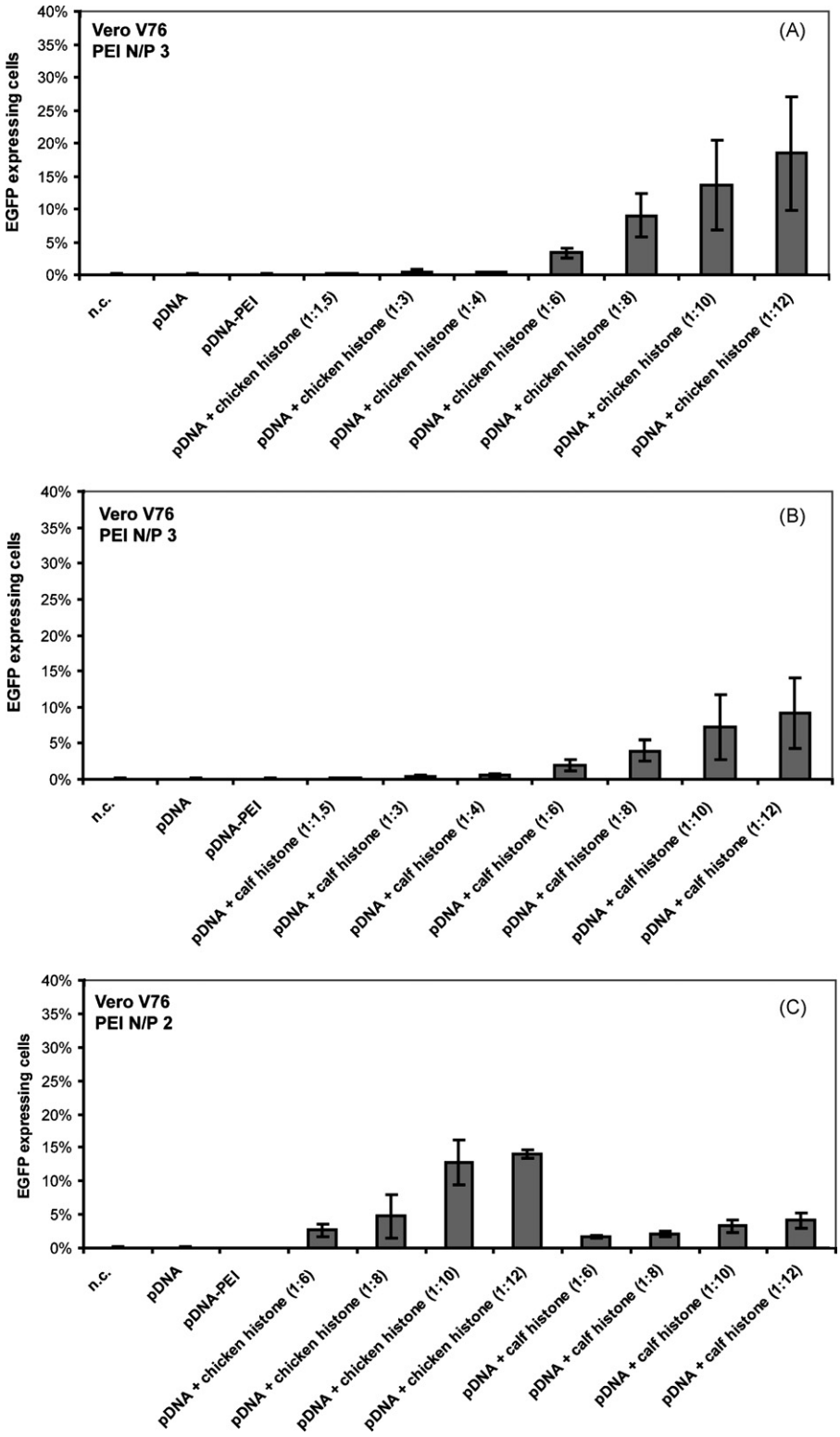

Fig. 4. Transfection efficiencies of pDNA/H–P particles in Vero V76 cells. EGFP expression was determined by flow cytometry 2 days post-transfection and compared to nontransfected cells (n.c.), cells transfected with naked pDNA and pDNA–PEI particles (N/P ratios 3 or 2), respectively. (A) pDNA/H–P particles composed of the indicated amounts of histones from chicken erythrocytes and PEI (N/P ratio 3). (B) pDNA/H–P particles composed of the indicated amounts of histones from calf thymus and PEI (N/P ratio 3). (C) pDNA/H–P particles containing histones, chicken or calf type, and PEI (N/P ratio of 2). Mean values of 3–5 experiments, error bars represent SD.

In summary, both Vero V76 and BHK-21 cells were accessible for transfection with pDNA/H–P particles. This effect was dependent on the origin of histone extracts, chicken or calf type, and PEI concentration used in the specific cell line: particles composed of pDNA, chicken histones and PEI at an N/P ratio of 3 were most efficient in the Vero V76 cell line. In contrast, particles with calf thymus histones (pDNA–histone ratio of 1:6 and 1:8) and PEI at a lower N/P ratio of 2 led to the best EGFP expression in BHK-21 cells.

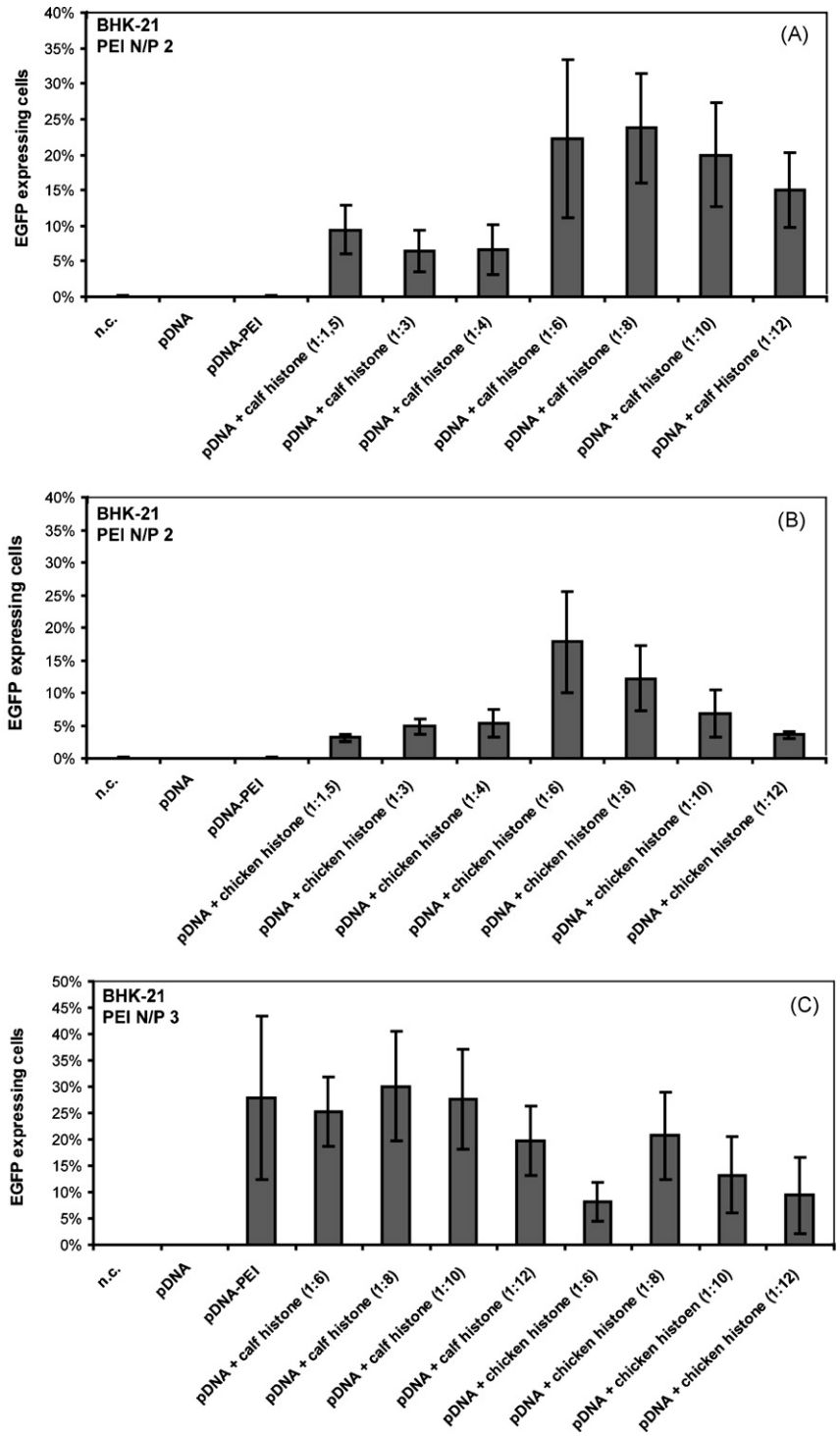

Fig. 5. Transfection efficiencies of pDNA/H–P particles in BHK-21 cells. EGFP expression was determined by flow cytometry 2 days post-transfection and compared to nontransfected cells (n.c.), cells transfected with naked pDNA and pDNA–PEI particles (N/P ratios 3 or 2), respectively. (A) pDNA/H–P particles composed of the indicated amounts of histones from calf thymus and PEI (N/P ratio 2). (B) pDNA/H–P particles composed of the indicated amounts of histones from chicken erythrocytes and PEI (N/P ratio 2). (C) pDNA/H–P particles containing histones, chicken or calf type, and PEI (N/P ratio 3). Mean values of 3–5 experiments, error bars represent SD.

Here, usage of histone concentrations higher than 1:8 decreased the transfection efficiency again. The reasons for this still have to be determined.

3.4. In vitro DNA tracking

We further investigated the finding that the addition of minimal concentrations of PEI to pDNA–histone complexes promotes the transfection efficiency, whereas histones or PEI alone were ineffective. After labelling pDNA with a fluorescent dye, the complexes were studied microscopically. As no intracellular particles were observed within the first hours of transfection (data not shown), and as best transfection efficiencies were obtained after 48 h, DNA tracking analysis was also done 2 days post-transfection. In addition, flow cytometry was performed to quantify the amount of cells that contained pDNA. For both analyses

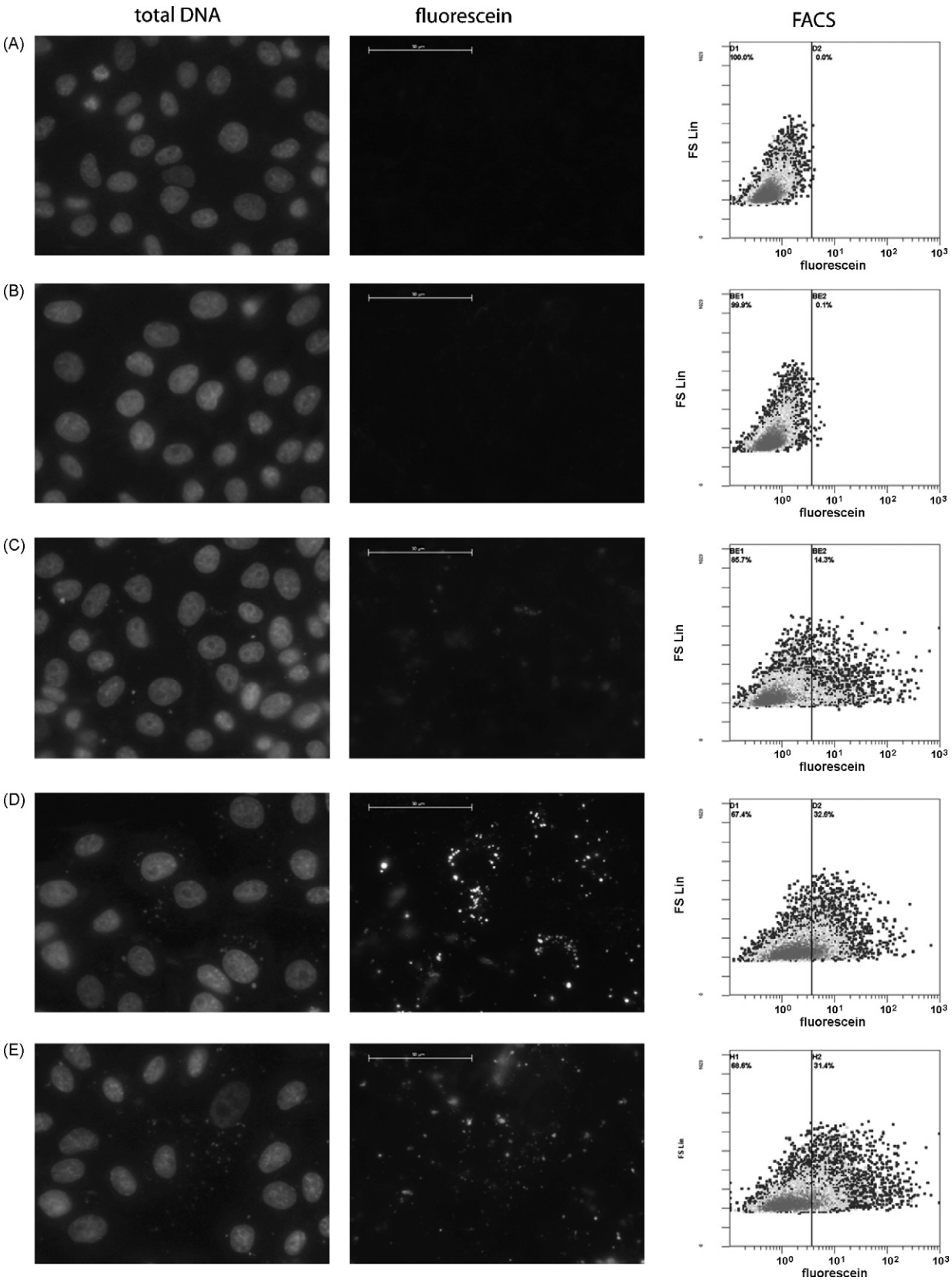

Fig. 6. Fluorescence microscopy and flow cytometry of Vero V76 cells transfected with fluorescein-labelled pDNA. Cells were analysed 48 h post-transfection. Left column, cells were stained with DAPI to visualize total DNA. Middle column, detection of intracellular fluorescein-labelled pDNA. Right column, flow cytometry of the cells, measuring fluorescein. Cells were incubated with: A, only medium; B, labelled pDNA; C, labelled pDNA–PEI particles; D, labelled pDNA–histone particles; E, pDNA/H–P particles.

surface-bound particles were removed. As can be seen in Fig. 6 (panel B), no signal was detected in cells incubated only with naked DNA equal to nontransfected cells (panel A). Few particles with low fluorescent signals were detected in cells transfected with complexes of labelled pDNA and PEI alone. 14.3% of the cells were measured as fluorescent by flow cytometry (Fig. 6, panel C). This indicates that pDNA–PEI particles, although they do not mediate gene expression with the low amount of PEI used, are capable to enter the cells. 32.6% of the cells incubated with pDNA–histone complexes contained pDNA as determined by flow cytometry (Fig. 6, panel D). The pDNA appeared as clearly visible dots in the cytoplasm, which might represent single particles or, alternatively, accumulations of the complexes (e.g. in endosomes). pDNA/H–P complexes were detected as intracellular dots (Fig. 6, panel E). The quantities of dots localised within the cells were comparable to pDNA–histone complexes, and also the flow cytometry results were similar (31.4% positive fluorescent cells). After co-staining lysosomes and nanoparticles, most if not all pDNA–histone complexes appeared to be associated with lysosomes (Supplementary Figure 1). Such a colocalisation was somewhat less pronounced with pDNA–PEI and pDNA/H–P particles.

3.5. Effects of the pDNA/H–P particles on cell viability

Next, it was investigated whether pDNA/H–P particles would show any toxicity to the cells. Both Vero V76 and BHK-21 cells were incubated with the particles as described above. Analysis of cell survival was done 2 days post-transfection by counting of viable cells (Fig. 7). Survival of Vero V76 cells transfected with naked pDNA or PEI at an N/P ratio of 3 was equal to nontransfected cells and cells incubated with pDNA/H–P particles (Fig. 7A). Usage of PEI at a higher concentration (N/P 5) resulted in slightly, but not significantly, decreased cell numbers. Similarly, no toxicity was observed in BHK-21 transfected with pDNA/H–P particles at pDNA–histone ratios of 1:8 or 1:10 and PEI (N/P 2) (Fig. 7B). Transfections with pDNA/PEI (N/P 5) complexes clearly decreased the viability of BHK-21 cells.

3.6. Size measurement of pDNA/H–P particles

To further characterise the pDNA/H–P particles we determined their average size and compared them to pDNA–histone complexes. Results are summarized in Table 1. For pDNA/H–P particles, a mean size of 1.9 μm was determined. Similar values were obtained with

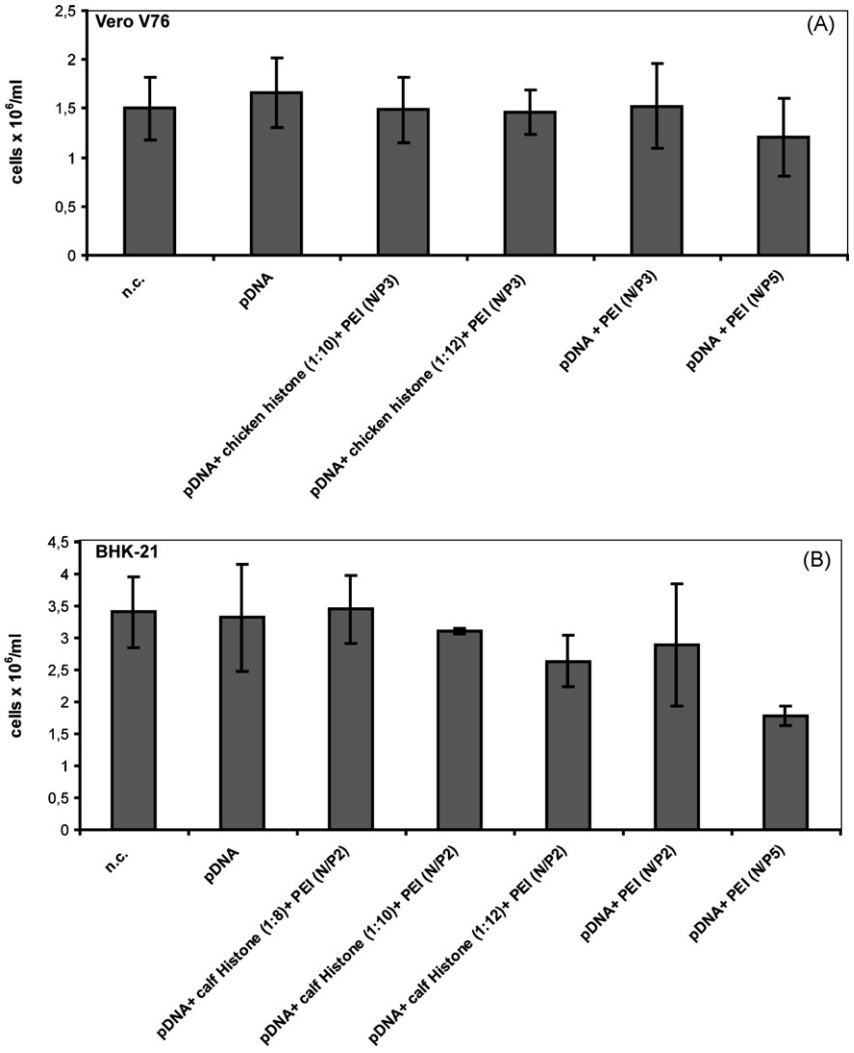

Fig. 7. Cytotoxicity studies of different pDNA/H–P particles analysed 2 days post-transfection by determination of viable cells numbers. (A) Vero V76 incubated with particles containing histones from chicken erythrocytes and PEI at an N/P ratio of 3. (B) BHK-21 cells incubated with pDNA complexed with histones from calf thymus and PEI (N/P 2). Cell numbers were compared to nontransfected cells (n.c.), cells transfected with naked pDNA and pDNA–PEI particles (N/P 2, 3 and 5), respectively. Mean values ± SD of 3–4 experiments.

Table 1
Dynamic light scattering experiments for size measurement of the various pDNA-bound particles. pDNA/H–P particles (A) were compared to particles composed of pDNA and PEI (B), pDNA and histones (C) and pDNA condensed first by PEI and subsequently by calf histones (D).

Type of particle	Mean size (nm) ± SD
A: pDNA/H–P (at N/P 3)	1868 ± 58
B: pDNA–PEI (at N/P 3)	2128 ± 210
C: pDNA–histones	378 ± 34
D: pDNA/P–H (at N/P 3)	3856 ± 204

pDNA–PEI particles. In contrast, pDNA–histone complexes were significantly smaller (0.33–0.4 μm).

In addition, the size of particles assembled by complexing pDNA first with PEI and subsequently with histones (pDNA/P–H) was measured. With a mean value of 3.8 μm, these particles were even larger than pDNA/H–P particles, which indicates that histones were able to interact with preformed pDNA–PEI complexes.

We were unable to determine the zeta potentials of these particles, which might be caused by their irregular surface and their large size.

4. Discussion

In this work we have investigated whether natural histone mixtures, isolated from calf thymus and from chicken erythrocytes, would be functional in non-viral gene delivery. In contrast to approaches using single recombinant histone proteins as transfection reagents (Fritz et al., 1996; Balicki et al., 2002), we hypothesized that the usage of natural histone mixtures would allow synergistic effects between the different proteins. In addition, no recombinant protein technology is required, which facilitates the preparation of the reagents. However, although the histone mixtures efficiently complexed DNA and were taken up by the cells, they were very ineffective in mediating expression of the gene encoded by the pDNA. Very large amounts of histones were needed to see an effect.

Transfection efficiencies significantly increased when the histone mixtures were used in combination with low amounts of PEI, a polymer which previously was successfully combined with DNA-interacting peptides in gene transfer studies, although at much higher concentrations (N/P ratios of 10 and higher; Schwartz et al., 1999; Shen et al., 2009). The effect observed in our study was synergistic, because neither PEI nor histones alone mediated any transfection under the conditions where a combination of both worked well. However, differences were observed between the two tested histone extracts resulting in a cell line specific transfection effect. Chicken histones together with PEI worked most efficiently in Vero V76 cells, whereas BHK-21 cells were better transfected by histones from calf thymus and PEI. Reasons for this still have to be determined. As very low amounts of PEI were used, the complexes were well tolerated by the cells.

Addition of PEI had a significant impact on the size of the complexes. Histones alone compacted pDNA to complexes of about 380 nm in diameter. In contrast, after adding PEI to the preformed pDNA–histone complexes, the particle size increased to almost 1.9 μm. Based on the change in particle size, we conclude that PEI interacts with the preformed pDNA–histone particles to form the pDNA/H–P particles. However, we cannot exclude that free PEI is left, which could also contribute the positive transfection effect seen with the pDNA/H–P particles (Boeckle et al., 2004). PEI alone assembled with pDNA to particles of similar size (ca. 2.1 μm). It is known that complexes consisting of PEI and DNA at low N/P ratios are large and become smaller when more of the polymer is added (Erbacher et al., 1999). Particle size can also be influenced by modification of assembly conditions, such as the buffer used (Goula et al., 1998). However, despite of the large size of the pDNA/H–P particles, they were endocytosed by the cells and successfully delivered the pDNA.

When the different particles were followed microscopically after pDNA labelling, it became clear that both pDNA–histone and pDNA/H–P complexes entered the cells within similar time spans and transferred similar amounts of DNA (as judged by microscopic images and flow cytometry results).

Therefore, the reason(s) for the lack of transgene expression using the pDNA–histone particles must be situated downstream of cellular entry. As an example, pDNA complexed by histones alone might not be released from the endosomes into the cytosol. Indeed, it has been shown that histones alone have a very poor potential to break endosomal membranes (Fritz et al., 1996; Zaitsev et al., 2002). Colocalisation experiments with nanoparticles and lysosomes tentatively support this model (Supplementary Figure 1). Supplementation of pDNA–histone complexes with minimal concentrations of PEI probably enhances endolysosomal release of the particles (Bieber et al., 2002). The NLS-containing histones, in turn, might stabilize the pDNA and direct its cytoplasmic migration towards the nucleus, thereby facilitating nuclear localization during cell division (Mesika et al., 2005). This model would explain why without histones, the low concentrations of PEI (N/P 2 and N/P 3) used in our study are not functional as gene carriers, which is in accordance to other investigations (Boussif et al., 1995; Pollard et al., 1998). Alternatively, in light of their smaller size compared to the pDNA/H–P particles, the pDNA–histone complexes might be too tightly compacted to mediate efficient release of the pDNA either in the cytosol or in the nucleus.

The data presented here indicate that histone mixtures purified from natural sources are inefficient gene carriers in vitro. However, they still can form potent transfection complexes when they are combined with minimal amounts of PEI. This finding might have consequences for potential in vivo gene delivery approaches. Due to the toxicity of PEI, it is essential that the amounts of this compound are low. Increasing the transfection effect by natural (even body-own) protein mixtures is a promising way to boost PEI-mediated gene therapies or DNA vaccines without increasing toxicity. Taken together, these results present a promising new strategy for non-viral gene delivery.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.ijpharm.2010.08.036.

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