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Research paper

Transfection of a mouse dendritic cell line by plasmid DNA-loaded PLGA microparticles in vitro

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Abstract

Targeting of DC for DNA vaccination may be achieved by DNA-loaded poly(lactide-*co*-glycolide) (PLGA) biodegradable microparticles, since DC efficiently capture these microparticles in vitro and in vivo. DNA was encapsulated in PLGA microparticles by spray-drying. Various additives were tested and process parameters adjusted in order to prevent degradation of the DNA during encapsulation. The highest degree of supercoiled DNA was maintained by adding a strong buffering agent, such as PBS or $NaHCO_3$, whereas the cryoprotective lactose did not show a significant protective effect. DNA-containing PLGA microparticles were administered to a mouse DC line. Transfection efficacy was compared with commonly employed cationic transfectants and was visually assessed by green fluorescent protein expression. Transfection rate was very low in DC for all microparticle formulations and was comparable with commonly used cationic transfectants. It is concluded that the transfection of DC using PLGA microparticles is feasible, but efforts need to be undertaken to improve transfection efficiency in vitro, which may in addition lead to improved immune responses in vivo.

Keywords: Poly(lactide-*co*-glycolide) (PLGA); Antigen-presenting cells; Transfection; Microparticles; Dendritic cells; DNA

1. Introduction

Dendritic cells (DC) are professional antigen presenting cells with a unique capacity to induce a potent immune response, and have thus been identified to play a crucial role in DNA vaccination [1]. Indeed, when suitably transfected, DC were shown to induce an immune response via T helper lymphocytes as well as cytotoxic T lymphocytes [2–6]. Inherent difficulty in transfecting DC, in combination with low numbers of DC found in the epidermis (about 1–3%) [7–10], is considered to be one of the major bottlenecks for this vaccination approach. DC were shown to be transfected in vitro by using adenoviral vectors [11,12], but severe safety concerns limit the use of viral vectors in humans. Alternatively, non-viral transfection means such as electroporation or DNA/liposome complexes lead to low transfection efficiencies [11,13,14].

Here we envision the design of microparticulate DNA vaccine delivery systems. Owing to the selective capability of DC to efficiently phagocytose particles in the micrometer range, that is shared with only few other cell types [15–21], DNA-loaded microparticles hold promise as targeted vaccine delivery systems. In fact, biodegradable microparticles prepared from poly(lactide-*co*-glycolide) (PLGA) have been recognized to induce an efficient immune response, both at the humoral and the cellular level [22–24]. In previous studies we demonstrated that PLGA microparticles are readily phagocytosed by monocyte-derived DC in vitro [17,18] and in vivo [25,26]. Nevertheless, the appeal of DNA-loaded biodegradable microparticles as a delivery system [17,27–29] is not only due to their potential to be taken up by phagocytosis, but also because a larger load of functional DNA can be delivered to DC via this route, whereas naked DNA is rapidly degraded in vivo [30]. In addition, encapsulation of DNA in biodegradable PLGA microparticles leads to a pulsatile release of DNA over an extended period of time that is not unlike the booster injections of a conventional vaccination cycle [31].

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In this study, we tested the transfection efficiency of DNA-containing microparticles in a DC line and compared it to commonly used cationic transfectants. Various formulations were prepared by spray-drying and checked for integrity of DNA upon encapsulation. Our results demonstrate that plasmid DNA can be efficiently preserved and protected upon encapsulation in PLGA microparticles. Nevertheless, with all of the studied microparticle formulations DC transfection rates were low and comparable to commonly used cationic transfectants.

2. Materials and methods

2.1. Materials

Poly(D,L-lactide-*co*-glycolide) (PLGA, Resomer RG502H, ratio lactide:glycolide 50:50, molecular weight 13,700, uncapped end groups) was purchased from Boehringer Ingelheim (Ingelheim, Germany). Polyethylenimine (PEI) (branched; MW 600,000–1,000,000) was from Fluka (Buchs, Switzerland). Green fluorescent protein (GFP) reporter gene plasmid was kindly provided by Mologen Holding AG (Berlin, Germany). Plasmid DNA was prepared using a Qiagen Giga Kit (Basel, Switzerland) according to the manufacturer's instructions and dissolved in nanopure water. Physiogel, a modified gelatine, was from Braun Biomedical (Emmenbrücke, Switzerland). Materials for cell cultures were purchased from Life Technologies AG (Basel, Switzerland). All other chemicals used were of analytical grade unless otherwise specified and purchased from Sigma (Buchs, Switzerland).

2.2. Preparation and characterization of microparticle formulations

DNA containing PLGA microparticles were prepared by spray-drying as described elsewhere [17,27]. Briefly, plasmid DNA, at a nominal loading of 4 μg DNA per mg polymer, was dissolved in water containing various additives (Table 1); 250 μl of the resulting solution was dispersed in 2.5 ml of a 5% (w/w) PLGA solution in dichloromethane by means of an ultrasonic processor (Vibra-Cell, Sonics and Material, Danbury CT, USA). The processor was equipped with a 6 mm microtip, and ultrasound frequency was 20 kHz. The output was at 40 W, and sonication was performed on ice for 10 s. The resulting w/o dispersion was spray-dried in a laboratory spray dryer (Model 190, Büchi, Flawil, Switzerland). The obtained microparticles were subsequently washed with a 0.1% (w/w) Pluronic F68 solution and water and dried under vacuum for 24 h. Size distribution of the microparticle formulations was measured by laser light scattering (Mastersizer X, Malvern Instruments Ltd.) and particle size was calculated based on Mie's theory [31].

Table 1
Effect of various additives on the stabilization of plasmid DNA upon microencapsulation in PLGA

Formulation	Additives to the DNA containing aqueous phase	Supercoiled DNA (%)[a]	
		DNA in o/w dispersion before spray-drying	DNA extracted from microparticles
EDTA–$NaHCO_3$	1 mM EDTA 0.1 M $NaHCO_3$	n.d.	53
EDTA–lactose	1 mM EDTA 300 mM lactose	85.8	10
EDTA–lactose–$NaHCO_3$	1 mM EDTA 300 mM lactose 0.1 M $NaHCO_3$	89.5	52
EDTA–lactose–PBS	1 mM EDTA 300 mM lactose PBS pH 7.4	86.6	52
Modified gelatine	10% modified gelatine	n.d.	33

[a] Content of supercoiled plasmid DNA was determined by agarose gel chromatography.

2.3. Integrity of DNA during the encapsulation process

To assess DNA integrity upon ultrasonication we used w/o dispersions containing plasmid DNA. Their composition was the same as of those used for spray drying (see above), but without polymer in the organic phase. Ultrasonication was performed under identical conditions as described above for the microparticle preparation and was executed for 10 s. After centrifugation, the upper phase containing plasmid DNA was withdrawn. DNA was analyzed by electrophoresis on a 0.6% agarose gel (Eurobio, Les Luis, France) for 90 min at 80 V/cm in a Tris-acetate–EDTA buffer system (pH 8.0), and DNA was visualized using ethidium bromide staining. The respective spots of supercoiled and open circular DNA were assessed for the relative content of supercoiled DNA by comparing spot intensity with a digital imaging software (Adobe Photoshop, Adobe Systems, San Jose, USA). Due to the more intense staining of open circular DNA compared to supercoiled DNA, the amount of supercoiled DNA may be underestimated.

2.4. Determination of DNA content in PLGA microparticles

After encapsulation, the microparticles were dissolved in dichloromethane, and DNA was extracted with Tris–EDTA buffer pH 8.0. DNA was then subsequently precipitated with 0.3 M sodium acetate and 2 volumes of 100% ethanol for 30 min at −20 °C. After centrifugation for 30 min at 4 °C, the supernatant was withdrawn, and the DNA dried and resuspended in nanopure water. DNA was analyzed on an agarose gel. The content of encapsulated DNA

and the presence of double-stranded DNA were assessed using the PicoGreen assay (Molecular Probes, Lucerne, Switzerland). Analysis of double-stranded DNA was performed in 96-well plates according to the manufacturer's instructions using an automated plate reader (FluoroCount, Canberra Packard SA) equipped with a filter set 485/530 nm.

2.5. DC cultures

Throughout the study we used an immortalized mouse DC line already described elsewhere (AG101) [32] (kindly provided by Dr M. Suter, Institute of Virology, University of Zurich, Zurich, Switzerland). The DC were grown in T-25 flasks in Dulbecco's modified Eagle medium supplemented with 10% FBS, 50 U/ml penicillin and 50 μg/ml streptomycin. Cultures were kept at 37 °C in a 5% CO_2 humidified atmosphere. Every third to fourth day, DC were passaged and diluted 1:10 in fresh medium in a new culture flask.

2.6. Transfection of DC by cationic DNA complexes

Prior to transfection, approximately 20,000 DC were seeded into 96-well plates. Twenty-four hours later, DC were transfected with plasmid DNA complexed to either dendrimeric Superfect (Qiagen) or liposomal CellFectin (Life Technologies AG) according to the manufacturer's instructions. DNA/PEI complexes were prepared by mixing DNA with the cationic polymer in PBS (pH 7.4) at a mass ratio of 1:2.5 [33]. For 20,000 DC, 80 ng of plasmid DNA were used. After 2 h, the medium was withdrawn and the DC were washed once with plain medium before adding complete medium. Expression of GFP was monitored for 6 days by fluorescence microscopy (filter: excitation 546 nm/emission 590 nm; Axiovert 35, Zeiss), and the total count of transfected cells per well determined.

2.7. Transfection of DC by PLGA microparticles

Prior to the addition of plasmid DNA-loaded microparticles, DC were seeded in 96-well plates (approximately 20,000 DC per well). Twenty-four hours later, either 5 or 10 μg of each DNA-containing microparticle formulations were added to the cells. The expression of the GFP reporter gene was monitored for up to 27 days by fluorescence microscopy and the total counts of transfected cells per well were determined.

3. Results and discussion

3.1. Integrity of plasmid DNA and characterization of microparticle formulations

Shear stress and acidic pH have been demonstrated to affect the integrity of plasmid DNA during encapsulation processes [17,27]. Extensive shear stress results in DNA fragmentation [27,34]. Acidic pH initially leads to single breaks in the DNA backbone which is followed by a transformation from supercoiled to open circular DNA [35,36]. Both topological forms of the DNA-supercoiled and open circular—are bioactive with the supercoiled form being more stable than the open circular form [37,38]. Finally, extensive exposure to acidic pH leads to complete degradation of biologically inactive DNA [27].

During encapsulation, plasmid DNA was exposed to shear forces resulting from ultrasonication of the w/o dispersion consisting of the aqueous phase containing DNA, and the organic phase containing PLGA [27]. Moreover, an acidic pH was reported for the aqueous phase after formation of the w/o dispersion, and in the solid core of PLGA microparticles upon water uptake during polymer degradation [17,27,39–41]. Therefore, we prepared various formulations and tested them for their ability to maintain the integrity of supercoiled plasmid DNA during the encapsulation process and in the microparticle's acid rich core (Table 1). The aqueous phase was buffered either with PBS or with $NaHCO_3$ to maintain a neutral pH during formation of the w/o dispersion and, in addition, to prevent the acidification of the microparticle's core during polymer decomposition. EDTA is a heavy metal ion chelator and was used as an additive to inhibit the enzymatic degradation of DNA by DNase. Upon its release from the microparticles EDTA is thus expected to reduce the enzymatic degradation of DNA after phagocytosis. Lactose was added as a cryoprotectant and suggested to reduce nicking of DNA, which may be due to the removal of water during the spray-drying process. Modified gelatine was successfully employed to protect protein therapeutics during the encapsulation process [42]. Proteins tend to bind to w/o interfaces, often a cause for their denaturation. Hence, protein denaturation may be prevented by an excess of modified gelatine which occupies the interface. However, due to the markedly hydrophilic character of DNA, we expect modified gelatine to protect DNA by its buffering capacity rather than by its occupation of the w/o interface.

As representative examples we tested the formulations EDTA–lactose, EDTA–lactose–$NaHCO_3$ and EDTA–lactose–PBS for DNA integrity during ultrasonication (Fig. 1, Table 1). The detrimental effect of ultrasound generally relates inversely to the exposed volume. Thus, to simulate the total volume of the w/o dispersion afterwards used for spray-drying, dichloromethane was added to the aqueous DNA solution. The polymer was omitted to allow easier separation of the DNA containing aqueous phase from the organic phase after ultrasonication, which would be problematic in the presence of the polymer.

Under the applied experimental conditions, ultrasound did not negatively affect the integrity of plasmid DNA. This excludes significant detrimental effects of ultrasound on the preparation of the w/o dispersion before spray-drying. The content of supercoiled DNA in control DNA was between 85.8 and 89.5%, and was fully preserved for up to 10 s

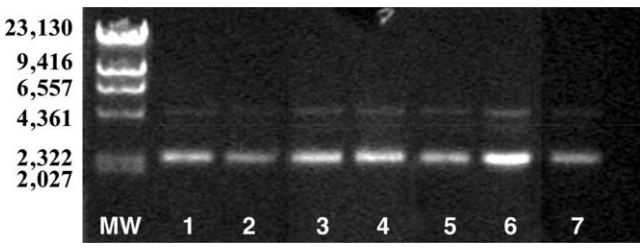

Fig. 1. Agarose gel showing the effect of ultrasonication (10 s) on plasmid DNA integrity. MW, λDNA/Hind III ladder; lane 1, aqueous phase of formulation EDTA–lactose before ultrasonication; lane 2, formulation EDTA–lactose after ultrasonication; lane 3, aqueous phase of formulation EDTA–lactose–$NaHCO_3$ before ultrasonication; lane 4, formulation EDTA–lactose–$NaHCO_3$ after ultrasonication; lane 5, aqueous phase of formulation EDTA–lactose–PBS before ultrasonication; lane 6, formulation EDTA–lactose–PBS after ultrasonication; lane 7, control DNA.

ultrasonication (Fig. 1, Table 1). Agarose gel chromatography revealed no noticeable low molecular weight fragments of the ultrasonicated plasmid DNA as compared to control DNA (Fig. 1).

In contrast, the preparation of the microparticles by spray-drying resulted in significant conversion of supercoiled plasmid DNA into the open circular form. Since ultrasonication of the w/o dispersion in the absence of polymer did not affect DNA integrity, this outcome could be either affected by the presence of the polymer and/or the evaporation of the solvents during the spray-drying process. Previously we have demonstrated the need for buffering additives in order to prevent detrimental effects of low pH values on plasmid DNA in the inner aqueous phase when dispersed in the organic solvent in the presence of PLGA [17]. Thus, we expected a beneficial effect on the preservation of supercoiled DNA when adding buffering additives (Table 1, Fig. 2). Nevertheless, although buffering

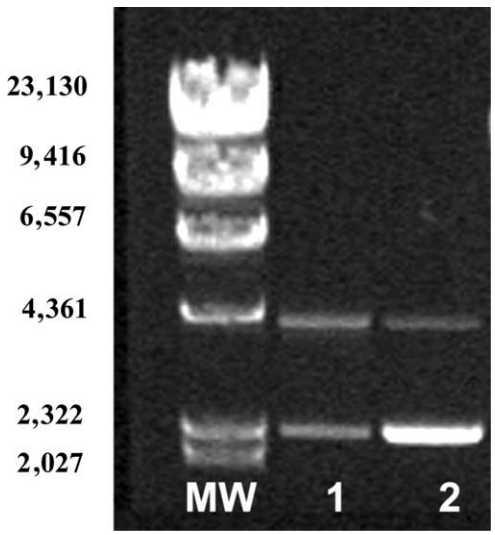

Fig. 2. Agarose gel showing the effect of encapsulation on plasmid DNA integrity in formulation EDTA–lactose as representative example. MW, λDNA/Hind III ladder; lane 1, DNA from microparticle formulation EDTA-lactose; lane 2, control DNA.

with $NaHCO_3$ was shown to maintain a neutral pH in the DNA-containing aqueous phase [17], nicking of DNA upon spray-drying was not entirely prevented. Rapid removal of solvents and microparticle formation during the spray-drying process could have further contributed to the loss of integrity. By adding lactose as cryprotectant, nicking of DNA during spray-drying was not noticeably prevented (Table 1), although lactose was previously demonstrated to be protective in experiments where freezing of the DNA-containing aqueous phase of the primary emulsion was performed before homogenization [41].

Among the various buffering additives, $NaHCO_3$ and PBS displayed equally protective efficacies and were superior to modified gelatine (Table 1). Combination of lactose with $NaHCO_3$ or PBS did not show any synergism (Table 1). In all formulations no noticeable amounts of low molecular weight fragments were detected by agarose gel chromatography (Fig. 2).

All formulations described in Table 1 were further analyzed for particle size distribution and encapsulation efficiency. As expected from previous experiments, the mean diameter of the various microparticle formulations ranged from 7.82 to 11.6 μm (Table 2) [27,33]. Loading of DNA, as detected by the amount of double-stranded DNA, was between 0.62 and 0.81 μg/mg with no noticeable difference between the various formulations (Table 2).

In summary, various biodegradable microparticle formulations were prepared which contain significant amounts of supercoiled and open circular plasmid DNA. Although supercoiled plasmid DNA has been found to be more stable against enzymatic degradation, supercoiled as well as open circular DNA were demonstrated to be effective in the transfection of cells [17,38].

3.2. Transfection of a DC line by cationic DNA complexes

In previous studies, we observed the start of in vitro release of bioactive DNA from PLGA microparticles on the second day after dispersion of the microparticles in the aqueous release medium [17,43]. Here we tested the efficacy

Table 2
Determination of plasmid DNA content and integrity after microencapsulation in PLGA

Formulation	Mean diameter (μm)	DNA content (μg/mg)[a]	Encapsulation efficiency (%)[a]
EDTA–$NaHCO_3$	11.6	0.81	20.4
EDTA–lactose	7.82	0.62	15.5
EDTA–lactose–$NaHCO_3$	10.29	0.77	19.3
EDTA–lactose–PBS	9.57	0.76	19.0
Modified gelatine	9.83	0.68	17.1

[a] Plasmid DNA was extracted from the microparticles as described in Materials and Methods and quantified by using a PicoGreen assay.

of such DNA-containing microparticles to transfect a DC line culture. As compared to other cell types, such as COS-7 cells (monkey kidney fibroblasts) where comparable amount of DNA/cell results in cellular protein levels of 84% (Superfect) and 82% (CellFectin) of those in control wells [44] or 293 cells (embryonal kidney cell line) where comparable amount of DNA/cell results in up to 10% of transfected cells [33], transfection of DC by more conventional approaches, e.g. electroporation, cationic molecule/DNA complexes or liposomes/DNA complexes, was also poor [11,45].

To estimate the efficacy of DNA-containing biodegradable microparticles to transfect the DC, we compared our formulations with commonly accepted and well characterized transfectants. Cationic complexes of various nature were formed with plasmid DNA by using branched PEI [46], fractured dendrimers (Superfect) or cationic liposomes (CellFectin). An optimized ratio of DNA to transfectant was used according to the manufacturer's instructions and based on previous experiments [33]. The amount of transfectant, respectively, DNA administered to the cells was chosen in order to be non-toxic for the cells (<5% necrotic cells) and is based on previous investigations in phagocytic cells [33]. A noticeably elevated number of cells floated in the medium after administration of PEI-DNA complexes. This cannot be explained to date and needs further investigation.

We employed an immortalized DC line, AG101, derived from the skin of mice, lacking both type I and type II interferon receptors [10,32,47]. The cells were shown to express constitutively CD45, CD11b, MHC class II, F4/80, N418, CD86 and ICAM-1, and were able to take up, process and present antigens to T cells. Furthermore, the DC were able to produce IL-6 and IL-12p40 similar to mature wild type DC. Therefore, AG101 cells demonstrate structural and functional wild type-like DC features and appear appropriate for the study of phagocytosis and transfection in vitro.

Whereas the various cationic transfectants have been previously shown to efficiently transfect several cell lines in our laboratory [33] and by others [44], the transfection rates of AG101 DC were extremely low with only about one transfected DC per 10,000 cells (Table 3, Fig. 3). No significant difference was observed among the different transfectants. Nevertheless, the episodic character of the in vitro transfection of DC, as observed in our study, does not preclude the efficient immune protection found in vivo [5].

By comparison of the non-viral DC transfection efficiencies achieved in our study with those observed by others, it is striking that our study resulted in particularly low transfection rates. Liposomal transfection or electroporation was reported to be highly variable resulting in rates of up to 10% of transfected monocyte-derived DC [11,14,45,48]. In the same cells efficiencies approaching 60% were obtained by so-called nucleoporation, an electroporation-type technique to deliver plasmid DNA directly into the nucleus [49]. Among DC of different origin, low transfection (<2%) was demonstrated in monocyte-derived DC as compared to CD34 + progenitor-derived DC with transfection rates of about 12% [13]. In addition to cell-type- and method-dependent variability, the reporter gene type and methodology used to assess transfection efficiency may also contribute to the observed variations. We cannot exclude that the GFP protein used in our study to visually assess the number of transfected DC is rapidly degraded, processed and presented on MHC class I or II after successful transfection. Thus, detection of GFP gene expression may be limited by a short half-life of the protein in the cells. Indeed, it was demonstrated by others, that even upon minor or absent transfection the protein was efficiently expressed, processed and presented to T cells in vitro [14,50] and in vivo [6]. Moreover, RT-PCR on mRNA was demonstrated to be more sensitive to detect evidence of gene expression after transfection than the occurrence of the intact protein [50].

Table 3
Transfection efficiency of DC with cationic DNA complexes

Day	Number of transfected DC[a]		
	Superfect	CellFectin	PEI
1	0	1	0
2	3	1	1
3	3	0	4
6	0	0	0
Sum	6 out of 60,000	2 out of 60,000	5 out of 60,000

[a] Transfected DC out of 3 wells total (approximately 20,000 DC/well). The numbers indicated represent transfected cells out of the estimated initial seeding. Transfection of DC was investigated over 6 days by fluorescence microscopy and total amount of transfected cells may include several countings of individual cells.

3.3. Transfection of a DC line by PLGA microparticles

The rationale of using microparticles as gene delivery vehicles is based on the selective propensity of phagocytes, such as DC, to readily internalize particles in the micrometer range [17,18,25,26]. This was expected to lead to the selective delivery in vivo of large amounts of encapsulated plasmid DNA to phagocytic cells, such as DC, where these cells are co-localized with various other cell types. In contrast, the most common transfectants do not selectively target DC, but transfect a broad range of cell types [44].

Either 5 or 10 μg of the various microparticle formulations containing either 3.1–4.0 or 6.2–8.0 ng plasmid DNA, respectively, were added to 20,000 DC, and the expression of GFP was monitored over a time period of 27 days. Previous reports demonstrated the release of bioactive DNA from PLGA microparticles over a time period of about 4 weeks [17]. The PLGA microparticles were efficiently phagocytosed by DC, from where they disappear rapidly through biodegradation within 1 week (Fig. 4) [17].

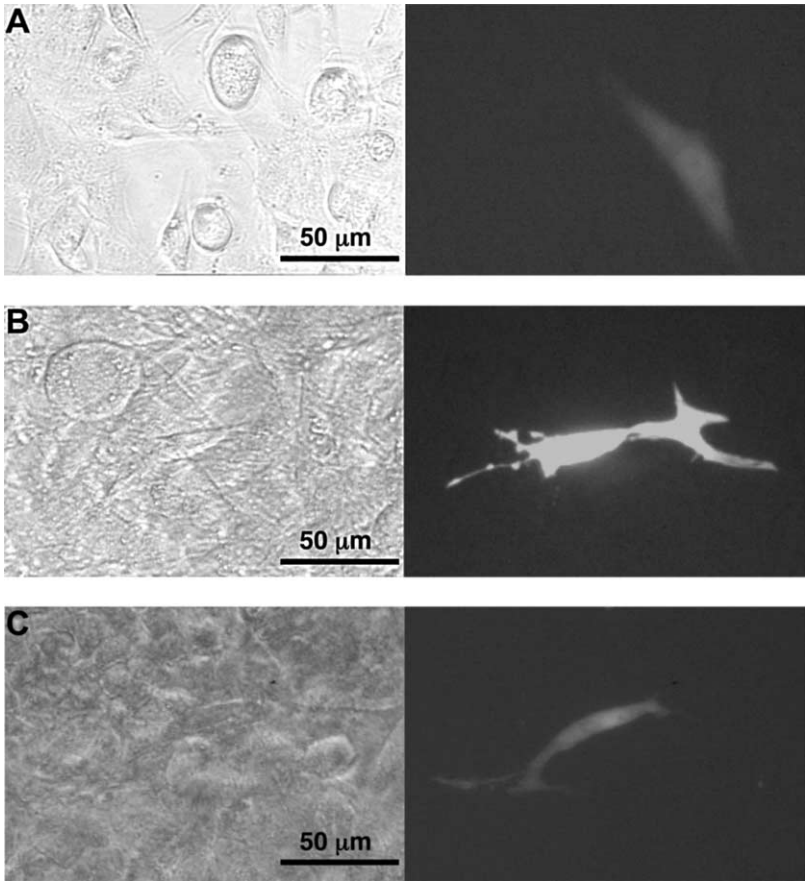

Fig. 3. Transfection of DC using Superfect (A), CellFectin (B) or PEI (C). DC were observed by fluorescence microscopy and pictures were taken at different time-points after transfection (Superfect 72 h; CellFectin 48 h; PEI 72 h).

All microparticle formulations, except for EDTA-lactose-PBS, displayed a small number of transfected DC over time (about 2–18 transfected cells per 80,000 cells; Table 4). Transfection efficiency was minor but in the same range as obtained with the cationic transfectants. The total amount of DNA administered to the DC was roughly the same for the various microparticle formulations, but about 10–20 times lower as compared to the amount used with cationic transfectants. There was no noticeable correlation between the number of transfected cells and the amount of supercoiled DNA in the microparticles. Apart from formulation EDTA–lactose–PBS with no detectable transfection, the lower dose of 5 μg microparticles per 20,000 cells tends to lead to slightly higher counts of transfected cells as compared to a dose of 10 μg microparticles per 20,000 cells. The dose of 5 μg of microparticles has been proven in previous studies to display only minor toxicity on phagocytic cells (<5% necrotic cells) [33]. The higher dose

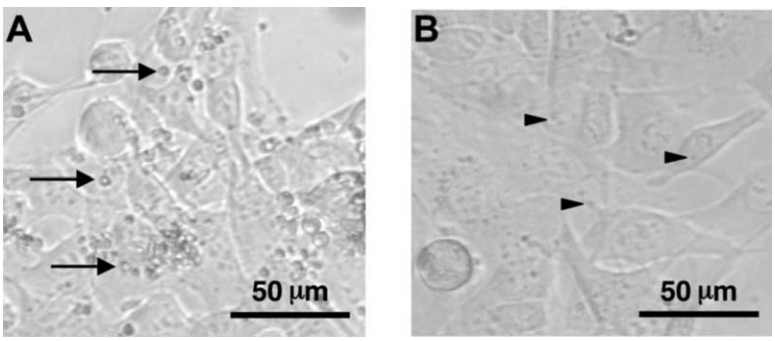

Fig. 4. Internalized DNA-loaded PLGA microparticles as detected in DC at 48 h (A) and 7 days (B) after administration. The arrows indicate microparticles in DC. Only minor remnants of microparticles remain after 7 days (B; arrowheads).

Table 4
Transfection efficiency of DC with DNA-loaded microparticles

Week	EDTA–$NaHCO_3$		EDTA–lactose		EDTA–lactose–$NaHCO_3$		EDTA–lactose–PBS		Gelatine	
	5 μg[a]	10 μg[a]	5 μg[a]	10 μg[a]	5 μg[a]	10 μg[a]	5 μg[a]	10 μg[a]	5 μg[a]	10 μg[a]
1	3	0	0	0	0	0	0	0	1	1
2	5	0	5	0	5	0	0	0	4	9
3	6	2	0	0	6	0	0	0	0	5
4	1	0	0	0	3	0	0	0	0	3
Sum	15	2	5	0	14	0	0	0	5	18
	cells out of 80,000 cells		cells out of 80,000 cells		cells out of 80,000 cells		cells out of 80,000 cells		cells out of 80,000 cells	

[a] Transfected DC out of 4 wells (approximately 20,000 DC/well initial seeding). A total amount of 5 and 10 μg of the various microparticle formulations containing 3.1–4 and 6.2–8 ng, respectively, was added to DC. The number of transfected cells was counted each day and was summed up for weekly recordings followed by the total sum after 27 days of counting. Thus, the numbers for weekly counts and total sum of transfected DC may include several countings of individual cells.

of 10 μg of microparticles was added in order to maximize the amount of DNA administered to the cells bearing in mind a slightly increased toxicity (5–10% cells) [33]. Thus, the trend towards slightly lower transfection rates by using a higher dose of microparticles may be accounted by an increase in toxicity.

Interestingly, most of the microparticle formulations showed delayed transfection starting at day 7 in contrast to cationic transfectants, where early transfection of DC was found (Table 3). This could be due to the typical bioerosion and release profile of PLGA microparticles in DC within 2 weeks [17]. In fact, phagocytosed PLGA microparticles are degraded in phagosomes [51] and, therefore, plasmid DNA must escape the phagosomes in order to be transcribed by the cellular machinery. This could explain the delay in GFP expression. Since non-phagocytosed microparticles were not removed and remained in the cell culture medium during the test, release of free plasmid DNA into the cell culture medium cannot be excluded and could have led to secondary transfection of other DC.

In vivo experiments performed by others showed therapeutic efficacy of similar DNA-loaded PLGA microparticles by demonstrating measurable immune responses in mice [23,52,53]. Moreover, when injected subcutaneously in mice, DC containing phagocytosed PLGA microparticles were found in the spleen [25]. As discussed above, detection of GFP protein expression in DC may have been unfeasible due to rapid protein processing and/or degradation. Therefore, transfected DC may indeed contribute to the immune response as observed in in vivo experiments [23,50,52,53], whereas continuous antigen production at the site of injection could be achieved by transfecting non-immune cells such as keratinocytes [1]. We observed large numbers of subcutaneously injected PLGA microparticles remaining at the injection site for about 1 week (unpublished results) while DNA might be released into the adjacent tissue. In mouse skin the incidence of DC is quite low with only about 900 DC present per mm^2 [54], accounting for only 1–3% of the total numbers of cells [55]. Taken together, these data indicate that at the site of injection transfection of non-phagocytic cells such as fibroblasts by released DNA is very likely to occur. This hypothesis is supported by a previous study showing that transfection efficiency in phagocytic cells is rather low as compared to non-phagocytic cells when using PLGA microparticles [33]. In other words, non-phagocytic cells could have been transfected by DNA-containing PLGA microparticles [33], and the thus transfected cells could then have produced and delivered the antigen to the DC [1].

Effective induction of the immune response does not require delivery and processing of the antigen, but also activation and maturation of DC [56]. Particulate DNA vaccine delivery systems ideally combine both features. Indeed, it was previously demonstrated by our group that plasmid DNA-loaded PLGA microparticles are able to induce activation and maturation of human monocyte-derived DC in vitro [43,57]. These findings support the potential of DNA-loaded biodegradable PLGA microparticles for potent immunostimulatory action.

4. Conclusion

To our knowledge, this is the first study to investigate the transfection of DC by using DNA-loaded microparticles. When comparing various formulations with commonly used cationic transfectants only very low transfection rates resulted with no noticeable differences between the various microparticle formulations and the cationic transfectants. Nevertheless, owing to the high immunostimulatory potential of DC, even low frequencies of transfected DC may be sufficient for vaccination purposes. In addition, DNA released from the microparticles could have caused the transfection of non-phagocytic cells and ensure the immune response. Overall, our data demonstrates potential to transfect DC by biodegradable microparticles, though at rather low rates.

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