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In vitro characterization and transfection of IL-2 gene complexes

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Abstract

Background: Interleukin-2 used in the treatment of malignant tumors has an anti-tumor efficacy. In this study, we have studied in vitro characterization and transfection efficiency of a plasmid encoding hIL-2, *pCXWN–hIL-2*, complexed to chitosan, polyethylenimine or DOTAP with varying ratios.

Methods: Plasmid DNA was amplified in *Escherichia coli* DH5α and isolated by alkali lysis method. The pDNA/chitosan, pDNA/PEI or pDNA/DOTAP complexes were analyzed by agarose gel electrophoresis for complex formation and by ESEM image analysis system for the morphology and DNA/medium relationship of complexes. DNase stability, the particle size and zeta potential values of complexes were determined. Transfection efficiencies of resulting complexes in two different cell lines were assayed by ELISA method.

Results: Conclusively, a transfection activity was observed in both cell lines (HeLa and Swiss3T3) with the order of pDNA/DOTAP > pDNA/PEI > pDNA/chitosan complexes. We have observed that the transfection efficiency was higher in HeLa cell line compared to Swiss3T3 cell line.

Conclusion: The physicochemical studies like stability, particle size and zeta potential, showed a relationship between the properties of a complex and its transfection efficiency.

Keywords: Interleukin-2; Chitosan; Polyethylenimine; DOTAP; Gene delivery; Non-viral

1. Introduction

It is known that interleukin-2 used in the treatment of malignant tumors has an anti-tumor efficacy (Rosenberg et al., 1990; Smith, 1984). However, its biological half-life is very short ($t_{1/2} = 13$ min) and hence frequent administration of the drug is necessary (Physician's Desk Reference, 2002). On the other hand, it results in the serious adverse effect of capillary leak syndrome including the side effects of pulmonary edema, hypotension and functional defects in organs and these serious side effects limit the use of IL-2. Conclusively, the efficacy of IL-2 treatment needs to be improved. Gene therapy, aiming the production of IL-2 in vivo by administering a vector encoding IL-2, seems to overcome those problems and promises to be an alternative for the protein therapy.

Gene therapy by non-viral vectors is safer though it is less efficient compared to viral vectors (Romano et al., 1999). In recent years, gene transfection with natural or synthetic cationic polymers or cationic lipids seems to be an attractive alternative due to the serious side effects associated with viral vectors even though they have a higher rate of a transfection capacity. Nevertheless, as the number of studies comparing the transfection efficiencies of cationic lipids and cationic polymers is limited and in most of such studies the reporter genes are used, the real situation of gene therapy with therapeutic genes remains blurred. Use of IL-2 plasmid DNA as a non-viral vector is at the preclinical and clinical level currently (Akbuğa et al., 2004). In clinical trials and animal studies, the plasmid DNA is formulated with a cationic lipid as the DNA carrier in order to increase the expression of IL-2 and attain a therapeutic level (Rosenberg et al., 1990; Smith, 1984).

In formulation studies with reporter genes, while some of the studies showed that DNA/cationic lipid complexes have higher transfection efficiencies (Felgner et al., 1987), the others showed that DNA/cationic polymer complexes have higher transfection efficiencies (Mumper et al., 1996; Tomlinson and Rolland, 1996). Chitosan and polyethylenimine are two of the promising

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DNA carrier materials to administer the DNA into the body for gene therapy (Boussif et al., 1995; Erbacher et al., 1998). In this study, we have searched whether the physicochemical properties, such as the ratio of the carrier material to the DNA encoding hIL-2, *pCXWN–hIL-2*, its particle size, zeta potential value, the morphology of the resulting complexes and the cell line, affect the transfection efficiency, while a comparison of cationic polymers and cationic lipids chosen as non-viral gene carriers was being taken into consideration. Chitosan and polyethylenimine have been chosen as cationic polymers and DOTAP^{TM} has been chosen as a cationic lipid to make complexes with the plasmid DNA. We have compared the transfection efficiencies of these complexes in vitro using HeLa and Swiss3T3 cell lines.

2. Materials and methods

2.1. Chemicals

Chitosan (M_W 150 kDa, 75–85% deacetylated) and branched polyethylenimine (PEI, M_W 25 kDa, a branched polymer) were purchased from Sigma (Darmstadt, Germany). 1,2-Dioleoyl-3-trimethylammonium-propane (DOTAP), DNase I (10,000 U/mg) and the ELISA assay kit for hIL-2 were purchased from Roche Biochemicals (Germany), *O*-nitrophenyl-β-D-galactopyranoside and β-galactosidase were purchased from Amresco (Ohio, USA). Fetal calf serum used was purchased from Biological Industries, Israel. All other chemicals were of pharmaceutical or molecular biology reagent grade.

2.2. Plasmid

The plasmid DNA, *pCXWN–hIL-2*, with 6300 bp size, constructed by Niwa et al. (Niwa et al., 1991) from the plasmid DNA *pCAGGS* with SV40 origin, CMV enhancer and a chicken β-actin promoter by inserting human IL-2 cDNA with 500 bp was used for the formation of complexes.

2.3. Preparation of plasmid DNA complexes

DNA/chitosan, PEI/DNA and DNA/DOTAP complexes were prepared by stirring two solutions, respectively. Chitosan was dissolved in 40 mM Tris–acetate (pH 5.0) at 0.005, 50 and 500×10^{-4}% final concentrations. DNA samples were dissolved in TE (Tris:EDTA, pH 8.0) buffer solution at 1–10 μg/ml. For a complex formation after stirring of two solutions, they were incubated for 30 min. Samples were prepared in triplicate. The resulting complexes were having the ratios of 1:0.005, 0.05, 0.1, 0.25, 1 and 2 (w/w) for DNA/chitosan, N/P 0.5, 1, 2, 3, 4, 6 and 10 for PEI/DNA and 1/1, 1/3 and 1/6 (w/v) for DNA/DOTAP complexes, respectively.

2.4. Electrophoretic analysis of plasmid DNA complexes

All three types of complexes were checked by agarose gel electrophoresis and the DNA was visualized under UV light as a result of intercalation of etidium bromide with the DNA. Complete complex formation resulted in retardation of the DNA in the loading well of the gel.

2.5. Environmental scanning electron microscopy (ESEM) of complexes

DNA/chitosan 1:1.5 (w/w) and PEI/DNA N/P 6 complexes were analyzed by Philips XL 30 ESEM-FEG image analysis system which is equipped with a field emission electron gun (BC stability < 1%/h, Schottky emitter) in ultra high vacuum (10^{-9} Torr) and an electron optical system, EDAX with high resolution (2 nm resolution) and a detector GSED. Samples were analyzed in solution in the sampler of the system with a pressure limiting aperture in vacuum (0.2–50 Torr). Samples were prepared in three parallel series in the experimental conditions being studied. Analyses were carried out by usual ESEM image creating methods and micro- and nano-particle size distribution, morphology and DNA–medium relationship characteristics of complexes were figured out.

2.6. Particle size and zeta potential measurement of complexes

Zeta potential values and particle sizes of DNA/chitosan, PEI/DNA and DNA/DOTAP complexes were measured by using a particle sizer and a zetameter (Malvern Instruments 3000 HS, UK). The instrument used is equipped with both a particle sizer and a zetameter unit. Helium neon laser unit of 10 mW produces a wavelength of 633 nm. The results have been assessed by Malvern PCS version 4.41 (1992) software. The samples was measured in glass cuvettes at 25 °C with a constant angle of 90°. Chitosan complexes were prepared in 40mM Tris–acetate solution at a pH of 5.0 having the codes of A0, A1 and A2. PEI complexes used in these experiment were having the codes of B0, B1 and B2 and the pH value of PEI complexes were 7.4 (see Table 1). DOTAP complexes were prepared at a ratio of 1/6 (w/v) and the pH of DOTAP solution was 6.2 as defined in the manufacturer's manual. The amount of DNA was 6 μg in each complex. Each measurement was done in triplicate with 10 different results. Zeta potential values of chitosan, PEI, DOTAP and DNA solutions were measured as well.

Table 1
Codes, zeta potential values and particle sizes of complexes ($n = 3$)

Code	Material	Zeta potential (mV ± S.D.) $n = 3$	Particle size (nm ± S.D.)
–	DNA	−18.30 ± 0.9	–
C1	Chitosan	58.50 ± 0.5	–
A0	DNA/chitosan 1:0.5 (w/w)	0.48 ± 0.7	11529.10 ± 1092
A1	DNA/chitosan 1:1 (w/w)	25.00 ± 1.3	415.95 ± 56.89
A2	DNA/chitosan 1:1.5 (w/w)	26.10 ± 0.6	493.52 ± 42.02
C2	PEI	36.40 ± 1.2	–
B0	PEI/DNA N/P 2	−8.90 ± 1.1	4287.26 ± 1032.89
B1	PEI/DNA N/P 4	21.00 ± 1.4	121.98 ± 3.62
B2	PEI/DNA N/P 6	32.00 ± 2.3	140.40 ± 3.62
C3	DOTAP	70.80 ± 7.4	–
D	DNA/DOTAP 1/6 (w/v)	36.20 ± 0.7	2900.20 ± 573.30

2.7. DNase I and serum stability of complexes

Stability of complexes against DNase I and serum with respect to time was analyzed. For DNase I stability study, complexes containing 6 μg *pCXWN–hIL-2* were reacted with 0.2 μl DNase I (2U), 40 μl DNase I reaction buffer (10×). Reaction was carried out at 37 °C and aliquots were taken at intervals 0, 2 and 24 h. For the inhibition of reaction 0.5 M EDTA was used. DNA degradation was analyzed with agarose gel electrophoresis as mentioned above and the experiment was repeated three times.

Stability of complexes against serum was studied by incubating the samples in 10% fetal calf serum and 150 mM NaCl solution at 37 °C. Samples were taken at intervals 0, 1, 24, 48 and 72 h and analyzed with agarose gel electrophoresis and the study was repeated three times.

2.8. Cell lines

HeLa (ATC # CCL-2) and Swiss3T3 (ATC # CCL-92) cell lines were used for transient transfection experiments. Both cell lines were maintained at 37 °C under 5% CO_2 (Heto-Holten Cell House 170, UK) in modified Eagle's medium (MEM) supplemented with 10% (v/v) fetal calf serum and penicillin (10,000 U/ml)–streptomycin (10 mg/ml)–amphotericin (0.025 mg/ml) mixture.

2.9. Transfection of cells and IL-2 assay

Cells were seeded in 24-well plate (5×10^4 cells/well) and grown at standard cell culture conditions for 24 h. Culture media were changed with fresh complete medium and complexes freshly prepared were added onto the cells. After 6 h of incubation, the complexes were removed, fresh complete medium was added and cells were incubated for further 48 h. After 48 h of incubation, ELISA for IL-2 was performed in the supernatant of the cell culture suspension, according to manufacturer's instructions. Briefly, streptavidin-coated microplates were washed with the solution containing anti-hIL-2 biotin. hIL-2 control serum solution, the standards and the cell culture supernatant were added to the microplates. Anti-hIL-2-peroxidase bound antibodies and the substrate (tetramethylbenzidine, TMB) solution were added. The change in the color of the solutions due to a chemical reaction was measured in an ELISA reader (Bio-Rad, USA) at 450 nm. Concentration versus absorption line was plotted with the standards of concentrations at 0, 23, 100, 170, 382 and 781 pg/ml. The concentration of each sample was calculated according to the line equation.

2.10. Cytotoxicity of complexes

In order to determine the effect of complexes on the viability of cells, cytotoxicity study with Trypan blue staining was performed (Ohashi et al., 2001). The cell suspension was diluted with Trypan blue solution 5% at a 1:1 ratio. The cells were grown for 72 h and then analyzed under microscope. The number of dead cells were calculated on a percentage basis.

2.11. Statistics

For the statistical evaluation of the results obtained from zeta potential, particle size measurement, IL-2 assay and cytotoxicity experiments, Student's *t*-test (one tailed, unequal variation type) was used. The variance values of the measurement groups with nonsignificat means ($p > 0.05$) were compared statistically by using *F*-test. It was accepted in both test types that the results are significantly different if $p < 0.05$.

3. Results and discussion

In this study, chitosan which is known to be biodegradable and natural cationic polymer (Lubben et al., 2001), polyethylenimine which is a synthetic and not biocompatible and having a rough correlation between toxicity and efficacy but known to have a high transfection capacity (Boussif et al., 1995; Godbey and Mikos, 2001) and DOTAP which is known to have better characteristics in regard to transfection compared to other cationic lipids (Li et al., 1999) have been complexed to a plasmid encoding IL-2 (*pCXWN–hIL-2*) and the transfection efficiency of the complex formed in this way has been measured in vitro.

3.1. Preparation of plasmid DNA complexes and control complex formation

Complex formation of DNA with chitosan, PEI or DOTAP has been controlled over an electrophoretic field. DNA/chitosan complexes were prepared with varying ratios and a complete complex formation was observed starting at a ratio of 1:0.25 (DNA/chitosan) (Fig. 1). In studies, chitosan complexes were generally prepared with respect to the ratio between free amino (N) and free phosphate in the DNA. However, the amount of free amino in the chitosan used in this study is not known previously, instead of N/P ratios, relative weight ratios were utilized in the preparation of complexes.

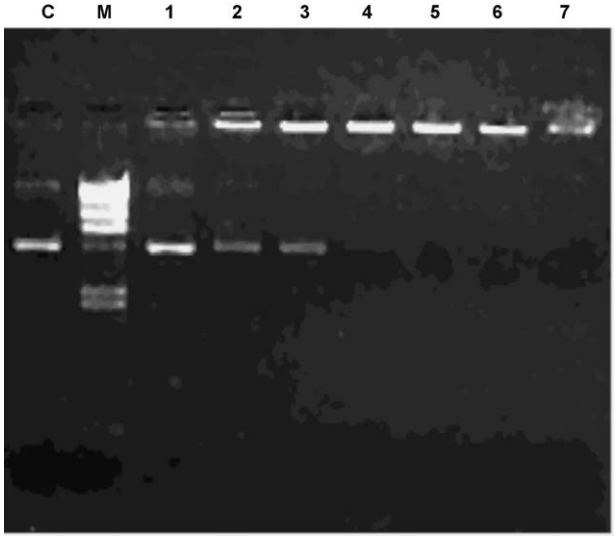

Fig. 1. Electrophoretic analysis of DNA/chitosan complexes. (C) *pCXWN–hIL-2*; (M) standard DNA (λ/HindIII); (lanes 1–7) *pCXWN–hIL-2*/chitosan complexes with ratios 1:0.005, 0.05, 0.1, 0.25, 0.5, 1 and 2 (w/w), respectively.

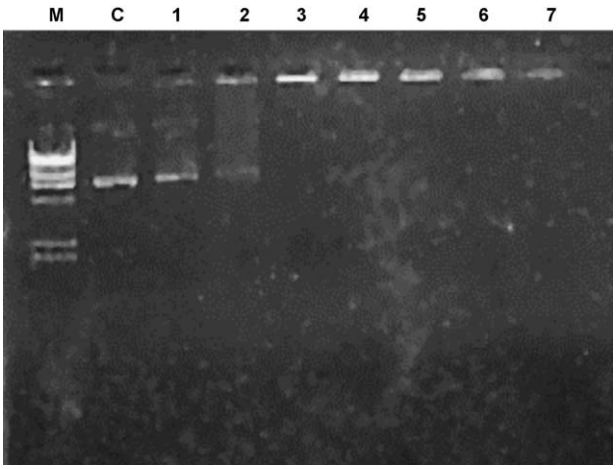

Fig. 2. Electrophoretic analysis of PEI/DNA complexes. (M) Standard DNA (λ/HindIII); (C) *pCXWN–hIL-2*; (lanes 1–7) PEI/*pCXWN–hIL-2* N/P 0.5, 1, 2, 3, 4, 6 and 10.

For the preparation of complexes with PEI, free amines (N) in the backbone of polyethylenimine and phosphate atoms in the backbone of DNA have been considered. It has been observed that the complexes were formed starting at N/P 2 ratio (Fig. 2). In other studies the basis for calculation of N/P ratios are varying and therefore different ratios of N/P for the complex formation is recorded in the literature (Choosakoonkriang et al., 2003; Kunath et al., 2003). Increasing the amount of PEI aggravates the intercalation of etidium bromide with DNA and it has been reported previously that complexes were hardly observed in the gel (Fischer et al., 1999). Similarly, in our study, the brightness of complexes decreased gradually after the ratio of N/P 6 due to the less intercalation of etidium bromide with the DNA (Fig. 2).

For the preparation of complexes with DOTAP, other than the ratio recommended by the manufacturer (DNA/DOTAP 1/6, w/v), smaller ratios were checked as well and it has been observed that the complex was formed starting at a ratio of DNA/DOTAP 1/3 (w/v) (Fig. 3). When DNA/DOTAP lipoplexes were treated with etidium bromide, although it intercalates with naked DNA, it is known that addition of DOTAP into the medium the florescence of etidium bromide decreases (Xu and Szoka, 1996). It has been observed in our study that due to the tight complex formation between DNA and DOTAP, etidium bromide could not intercalate with DNA well and the complexes after the ratio of N/P 3 were hardly observed (Fig. 3).

3.2. Environmental scanning electron microscopy (ESEM) of complexes

With ESEM-FEG&EDAX image analyzer system, the particle size, morphology and DNA–medium relationship of DNA/chitosan and PEI/DNA complexes were analyzed and it has been observed that DNA forms complexes with chitosan (Fig. 4) and PEI (Fig. 5). An analysis in wet mode has been utilized for the evaluation of images. Plasmid DNA, at the experimental conditions generated, has been observed with an appearance of a bright, rod-like and moving structure. Chitosan was having a smooth structure and interacting with DNA

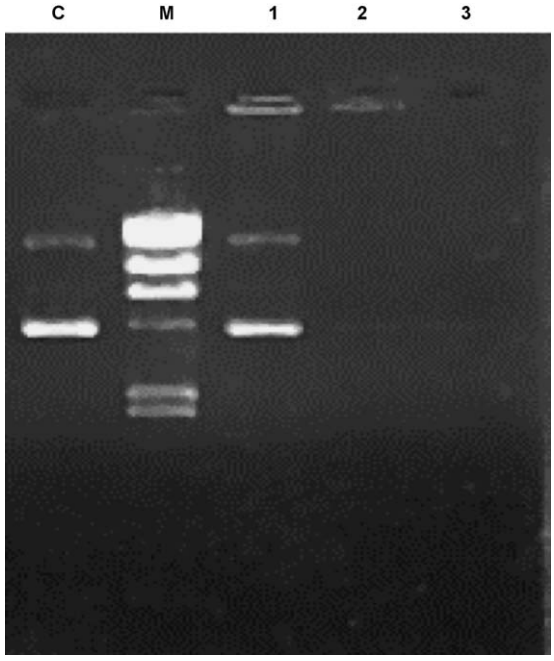

Fig. 3. Electrophoretic analysis of *pCXWN–hIL-2*–DOTAP complexes. (M) Standard DNA (λ/HindIII); (C) *pCXWN–hIL-2*; (lanes 1–3) DNA/DOTAP complexes with ratio 1/1, 1/3 and 1/6 (w/v).

at its different sites (Fig. 4). The particle size distribution of DNA/chitosan complexes, being in agreement with the results obtained using Malvern particle sizer, was heterogeneous with particle sizes smaller and bigger than 500 nm (Fig. 4). PEI has been observed as having a porous structure covering DNA from various sides (Fig. 5). There is no previous study reported that DNA/chitosan or PEI/DNA complexes were analyzed with ESEM-FEG image analysis system. However, it has been previously reported that morphological structure of chitosan and PEI complexes were analyzed with TEM, cryoTEM, SEM and fluorescence microscopy (Dunlap et al., 1997; Köping-Höggard et al., 2003; Lee et al., 2001). Köping-Höggard et al. (2003) have reported that Chitosan/DNA complexes were having toroidal and rod structure while DNA/PEI complexes were having a

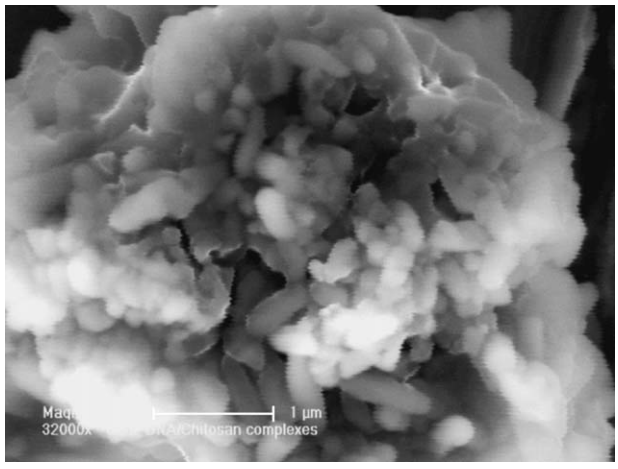

Fig. 4. Image of DNA/chitosan complexes obtained using with ESEM-FEG&EDAX image analysis system.

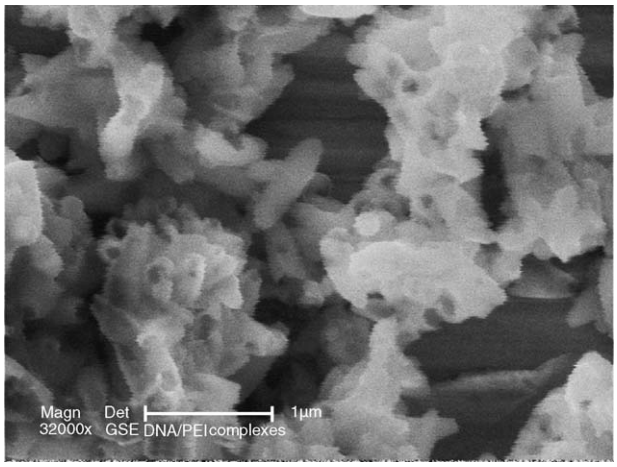

Fig. 5. Image of PEI/DNA complexes obtained using with ESEM-FEG&EDAX image analysis system.

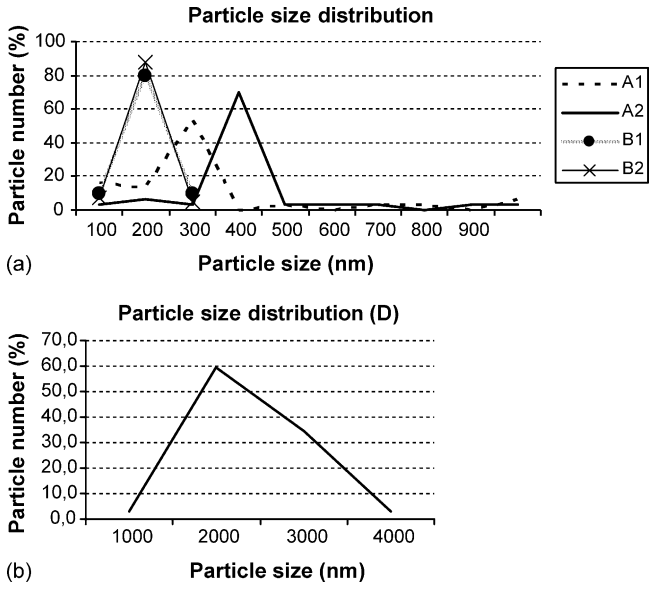

Fig. 6. (a) Particle size distribution of chitosan and PEI complexes. (b) Particle size distribution of DOTAP complexes.

globular structure as they observed in a study using TEM and cryoTEM microscope. Also, the results obtained using cryoTEM and TEM were in compliance with each other. The same research group (Köping-Höggard et al., 2003), in another study with florescence microscope reported that DNA/chitosan complexes were globular and like in our study, they formed complexes with DNA by interacting at its various sites. Dunlap et al. (1997) reported that they observed DNA/PEI complexes as globular, rod or toroidal structures in their analysis with SEM microscopy.

3.3. Particle size and zeta potential measurement of complexes

We have found that the average particle size of DNA/chitosan complexes is of around 500 nm (A1 and A2 as in Table 1). It has been previously reported that complexes having a particle size of approximately 500 nm and nano-particle of lower than 100 nm have successfully transfected the cells (Li et al., 2003). It has also been stated that the particle size depends on the ratio of N/P. While Erbacher et al. (1998) reported that increasing N/P ratio decreases the particle size, Ishii et al. (2001) reported that increasing N/P ratio increases the particle size. In our study, as seen in Table 1 and Fig. 6(a), the particle size of chitosan complexes with lower chitosan concentration (A1) was narrowly distributed compared to the chitosan complexes with higher concentration (A2) although it is not statistically significant ($p > 0.05$). In the study the zeta potential values of chitosan complexes used are of positive value as seen in Table 1 while the difference between zeta potential values of both complexes are statistically significant ($p < 0.05$). Increasing the amount of chitosan increases the zeta potential from 25 to 26 mV.

As in Table 1, the particle size of PEI/DNA complexes varies between 121 and 140 nm. Our findings are in compliance with studies performed with a reporter plasmid (Finsinger et al., 2000) showing that increasing N/P ratio increases the particle size. Although the average size of B1 and B2 complexes are different they both show a similarly homogenous distribution around 121–140 nm (Fig. 6(a)).

As shown in Fig. 6(b), DNA/DOTAP complexes were having the biggest particle size with an average of 2900.20±573.30 nm.

3.4. DNase I and serum stability of complexes

In this study, it has been observed that while the naked DNA degraded completely within the first 60 min with the effect of DNase I (Fig. 7(a)), complexes which showed a transfection efficiency in the cell culture (A2, B1, B2 and D) protected the DNA well against the degradative effects of DNase I (Fig. 7(b)). However, during the period the experiment was completed, there were differences among the complexes in regard to the stability. For example, the complex containing a lower level of chitosan (A1), with effect of DNase I degraded 30% within 120 min showing a 10% closed circular and 20% open circular form (data not shown).

In the presence of serum, DNA/chitosan complexes and DNA/DOTAP complexes relaxed at the end of 72 h, yet no DNA was released. On the other hand, PEI/DNA complexes protected the DNA tighter and no relaxation was observed (Fig. 8).

3.5. In vitro transfection of complexes

Having considered the results of physicochemical studies performed previously, two chitosan complexes (A1 and A2), two PEI complexes (B1 and B2) showing appropriate properties in terms of cell culture and DOTAP complex prepared according to the manufacturer's manual were chosen to be tested comparatively for their transfection efficiency in both cell lines. Complexes were prepared with plasmid DNA encoding human IL-2 (*pCXWN–hIL-2*). All complexes showed the highest level of transfection efficiency at 48 h with an order of the complexes tested: D > B2 > B1 > A2 > A1. DNA/DOTAP complexes showed the highest level of transfection activity while PEI complexes showed better transfection activities compared to chitosan

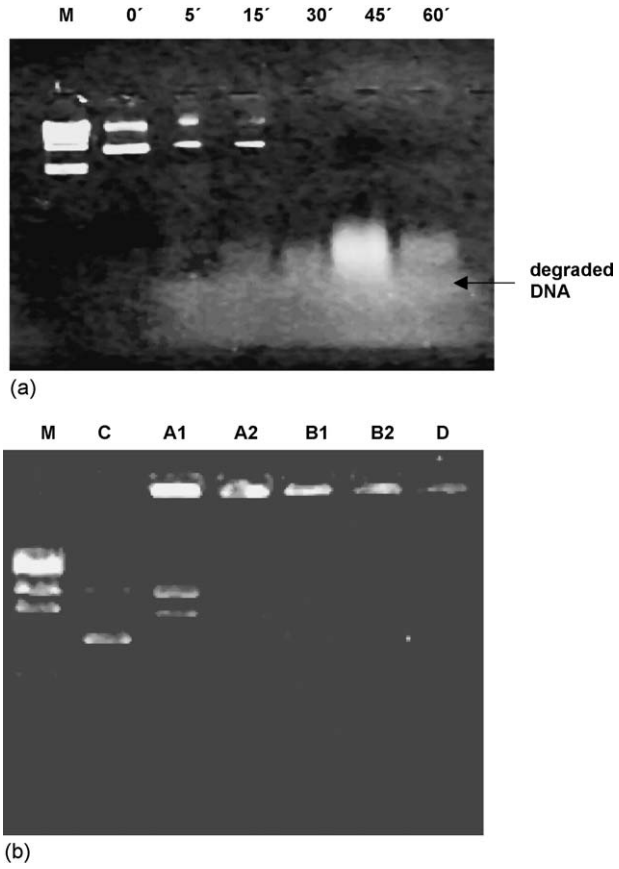

Fig. 7. Stability of complexes against DNase I. Panel (a) at the beginning of the incubation. (b) After 24 h of incubation. (M) DNA marker; (C) DNA control.

complexes. The order of D > B2 > B1 > A2 > A1 with respect to the transfection efficiency is the same in both cell lines studied, and the amount of hIL-2 expressed by using each complexes was higher in HeLa cell line ($p < 0.05$). The type of the cell line, HeLa cells originating from tumor cells and Swiss3T3 cell originating from normal embryo cells, may be playing an important role in this difference. Therefore, further similar studies should be performed in other types of cells.

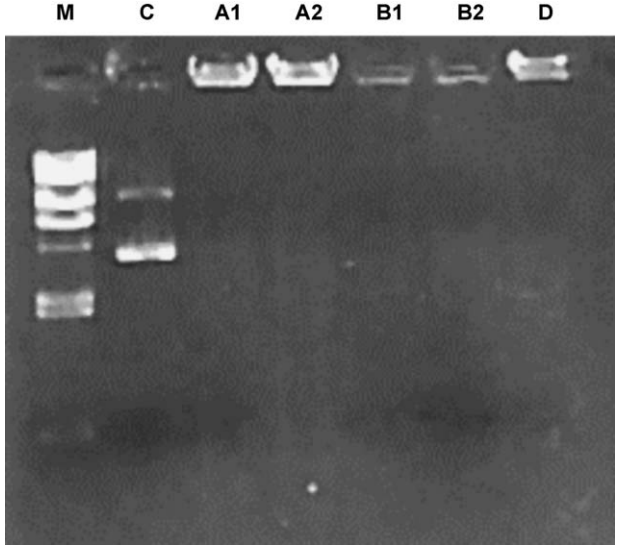

Fig. 8. Stability of complexes against serum after 72 h of incubation. (M) DNA marker; (C) DNA control.

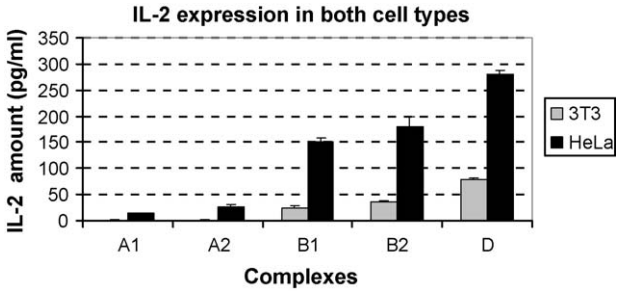

Fig. 9. IL-2 expression in HeLa and Swiss3T3 cell line (with 2 μg DNA, $n = 3$). Control was not shown due to the low level of absorption (IL-2 amount expressed in both cell lines = 1.15 ± 0.10).

The chitosan complex having a higher concentration of chitosan (A2) showed a higher level of transfection (Fig. 9). Erbacher et al. (1998) have previously reported that chitosan–DNA complexes containing even aggregates showed a higher level of transfection.

Regarding PEI complexes, even a slight increase in the size of complexes from 121 to 140 nm enabled an increase in the level of hIL-2 expressed from 151 to 180 pg/ml (Fig. 9). Likewise, zeta potential values of PEI/DNA complexes are 21 and 32 mV as seen in Table 1 and B2 complexes showed a higher level of transfection efficiency as seen in Fig. 9. A higher value of zeta potential can be relevant to the increase in the transfection efficiency. Rudolph et al. (2002) reported that the complexes they prepared by nebulisation retained its high zeta potential value (40–45 mV) and they showed a high level of transfection.

Similarly, large particle size and high zeta potential value of DNA/DOTAP complexes may be relevant to the high transfection capacity. It has been previously shown that the particle size and the zeta potential values of cationic lipid complexes are very important in their transfection capacity (Köping-Höggard et al., 2004). It is recorded in some studies that the zeta potential value of DOTAP lipoplexes are high (Zuidam and Barenholz, 1999). Similarly, in our study, as seen in Table 1, the zeta potential value and the particle size of DOTAP/DNA complexes is higher than the other complexes. It is recorded in previous studies that the mechanism of gene transfer by using DOTAP containing carriers is through membrane perturbation (Zuidam and Barenholz, 1999) and carriers having a lipidic structure showed a higher level of transfection in rapidly dividing cells like HeLa (Escriou et al., 2001).

Regarding the stability of the complexes, increasing the concentration of chitosan by two-fold protected the DNA better against the degradative effects of DNase I and serum, hence increased the amount of hIL-2 expressed from 13.98 ± 0.47 to 26.11 ± 4.06 pg/ml (Fig. 9).

3.6. Cytotoxicity of complexes

It is very well known that chitosan showed a low level of cytotoxicity. Due to its low level of cytotoxicity, chitosan has

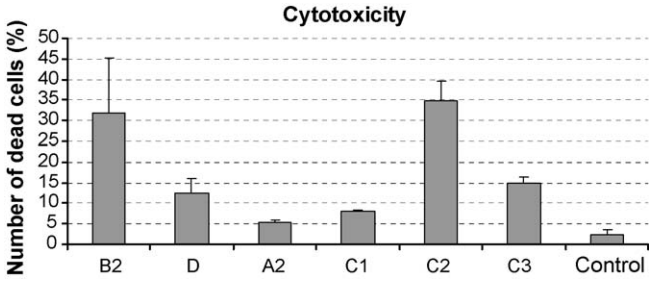

Fig. 10. Cytotoxicity of complexes and carriers at the end of 72 h of incubation. (C1) Only chitosan; (C2) only polyethylenimine; (C3) only DOTAP.

been particularly studied for gene transfer purposes (Köping-Höggard et al., 2001; Lee et al., 2001; Sato et al., 2001) and it has also been shown in our study that at the end of 72 h of incubation with carrier molecules either alone or in complex form with DNA, chitosan complexes were the least cytotoxic gene carrier material leaving only 5–10% dead cells (Fig. 10). It is recorded in many studies that complexes prepared with PEI are very toxic for the cells (Fischer et al., 1999; Kunath et al., 2003). It has also been showed in our study that PEI and PEI/DNA complexes are very toxic for the cells and the amount of dead cells at the end of 72 h has increased up to 40% (Fig. 10). Although it has been reported that cationic lipids are toxic for the cells (Lappalainen et al., 1994), DOTAP has shown to be relatively less toxic.

Conclusively, studies which are aiming to develop a gene carrier system for the expression of human IL-2 are very limited. In this study, we aimed to comparatively assess the physicochemical properties and their relation to the transfection efficiencies of three carriers, namely, chitosan, PEI and DOTAP, one of which is a natural polymer and the others are synthetic, as a gene carrier system for the expression of IL-2. To perform comparative studies with therapeutic genes in the treatment of somatic diseases with either viral or non-viral gene therapy methods is very crucial in order to reveal the efficiency of the treatment in vivo. Therefore, this study using, cationic polymers and cationic lipids which have been recently shown to be promising gene carriers studied comparatively in their transfection efficiencies, is very important in the constitution of a basis for further both in vitro and in vivo studies.

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