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N-Diethylmethyl chitosan for gene delivery to pancreatic cancer cells and the relation between charge ratio and biologic properties of polyplexes via interpolations polynomial

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ABSTRACT

In gene therapy of pancreatic cancer, non-viral vectors show an important role. These vectors are modified with the aim of improvement for pancreatic cancer gene therapy. For this aim, we used N,N-diethyl N-methyl chitosan (DEMC) for gene delivery to human pancreatic cancer cells (AsPC-1). pEGFP (Enhanced green fluorescent protein plasmid) was used as a model plasmid. In order to evaluate the efficiency of this polymer for gene delivery, the DEMC/pEGFP complexes are characterized via photon correlation spectroscopy, gel electrophoresis, fluorescence microscopy, flow cytometry and MTT assay. Also cancer cells' mean fluorescence intensity (MFI) and size changes after transfection are evaluated. The enhancement in polyplexes' charge ratios from 5 to 40, results in 16.70-fold increase in transfection efficiency. Higher MFI, cell size and cytotoxicity were observed as the N/P ratio increased. Considering that mathematical models can be used to understand and predict consequences associated with nanomedicine, the relation between DEMC/pDNA complexes charge ratio, cell transfection and toxicity was evaluated for the first time with Lagrange's interpolation polynomial method.

1. Introduction

Pancreatic carcinoma is the fifth leading cause of cancer-related death and up to now, the only curative treatment for pancreatic cancer is surgical resection. Conventional chemotherapy and radiotherapy approaches have been used to improve patient's survival but are relatively ineffective. Thus, to improve the prognosis, there is a need to develop a new modality of treatment for pancreatic cancer (MacKenzie, 2004). Advances in the molecular understanding of this malignant disease over the past years might lead to new treatment strategies. Strategies of gene therapy, antiangiogenic treatments, immunotherapy, and signal-transduction inhibition are in preclinical development (Beger et al., 2002).

For gene delivery to pancreatic cancer cells, several lipids and polymers have been used as non-viral vectors (Aoki et al., 2001; Miyata et al., 2008; Vernejoul et al., 2002; You et al., 2007). Among these vectors, chitosan is also considered to be a good candidate. However, low solubility and transfection efficiency has limited its application in cancer gene delivery in vivo. Therefore, researchers have modified this polymer in order to get an effective transfection. One of these modifications is quaternizing the polymer. According to studies on a quaternized chitosan, e.g. Trimethyl chitosan (TMC) (Germershaus et al., 2008; Kean et al., 2005; Thanou et al., 2002), by quaternizing the polymer, its positive charge, solubility, plasmid interaction and transfection are increased.

Recently mathematical biology has become very popular and in the last ten years the explosion in quantitative experimental data has meant that models are both more necessary and better validated. The mathematics in biology can be used for (i) understanding; (ii) prediction and (iii) guide specific nanoscale experiments to save time and resources (Johnston et al., 2007). Unfortunately, when it comes to modeling in the field of nanomedicine, the relation between accuracy and insight is very complex and difficult to maintain. The reason is due to the fact that for an effective nanomedicine, many physical and chemical interactions should occur on the nanometer scale and there are also biological processes, which occur on the micron scale (Robert and Freitas, 2005).

Obviously applying multi-scale mathematical techniques, in mathematical approaches, can extremely aid us in understanding nanobiosystems. However, multi-scale modeling is in its first stages and this is one of the primary challenges for mathematicians in

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order to explore the effects of nanoparticles in human body (Bewick et al., 2009). One of the multi-scale modeling techniques used in medicine is interpolations polynomial. Interpolation is a specific case of curve fitting, in which the function must go exactly through the data points. Interpolation polynomial is a generalization of linear interpolation and this interpolant is replaced by a polynomial of higher degree. Lagrange's interpolation method is a simple and clever way of finding the unique *k*th-order polynomial that exactly passes through $k+1$ distinct samples of a signal. Once the polynomial is known, its value can easily be interpolated at any point using the polynomial equation (Berrut and Trefethen, 2004).

N,N-Diethyl N-methyl chitosan (DEMC) was synthesized by partial quaternization of chitosan as described previously and has been proved to be an efficient tool for improving paracellular transport of hydrophilic drugs in the colon epithelial (Avadi et al., 2004). The polymer charge density was $21 \pm 5\%$. Its zeta potential was +58 mV. This modification renders the polymer solubility over a wider pH range. For its ability as penetration enhancer of poorly absorbable compounds, the polymer's efficiency for gene delivery to pancreatic cancer cells (AsPC-1) is evaluated and predicted.

2. Materials and methods

2.1. Materials

DEMC was synthesized from low molecular weight chitosan (Primex-Iceland). AsPC-1 cells from NCBI, Iran. Plasmid extraction (Fermentas Miniprep Kit, Lithuania), RPMI 1640 cell culturing medium (Gibco, BRL, UK), FBS (Fetal Bovine Serum, Gibco® Invitrogen Ltd., UK) and Arrest-in commercial transfection reagent (Open Biosystem, USA) were obtained from stem cell technology institute (Tehran, Iran).

2.2. Plasmid preparation

Briefly, after thawing Escherichia coli ($DH_{5\alpha}$), it was aliquot into pre-chilled microfuge tubes. Plasmid was added to cells and mixed. $DH_{5\alpha}$/plasmid mix were incubated on ice and heat shocked at 45 °C. LB (liquid broth nutrient medium) was added to the cells with shaking and then centrifuged. Transformed cultures were plated on LB containing ampicillin and incubated for 12–16 h. Cells were mixed with LB containing ampicillin and gently mixed at 37 °C. After 16 h the transformed bacteria were centrifuged and the plasmid was isolated via plasmid miniprep kit. The plasmid concentration (7 kbp) and purity were determined using BioPhotometer Eppendorf (Hamburg, Germany) and electrophoresis on 1.5% agarose gel. The gels were stained with ethidium bromide (10 μg/mL) and photographed on a UV transilluminator (Uvidoc, Bridgeville, UK).

2.3. Preparation and characterization of complexes using quaternized chitosan derivatives

DEMC was diluted to the respective concentration in serum free cell culture medium. To mimic conditions after polyplex formation and after addition of polyplexes to cell culture media, size and zeta potentials were determined in RPMI. DEMC–plasmid complexes were prepared by addition of the respective polymer solution to an aqueous solution containing 1 μg pEGFP at different N/P ratios (ratio of DEMC nitrogen (N) and phosphate (P) of DNA) from 5 to 10, 20 and 40. The solutions were pippetaged gently and shaken at 250 rpm for 30 min at room temperature. Arrest-in/plasmid (1 μg) complexes were prepared according to its protocol (Thermo Scientific Open Biosystems, Arrest-in Transfection Reagent, Catalog #: ATR1740, ATR1741, ATR1742, ATR1743). Particle size and the zeta potential of the nanoparticles were measured using photon correlation spectroscopy and Malvern Zetasizer (3000HS, UK).

2.4. Agarose gel electrophoresis

10 μl polyplex solution were mixed with plasmid prepared as described above and were applied to a 1% agarose gel in TAE (40 mM Tris/HCl, 1% acetic acid, 1 mM EDTA, pH 7.4) containing 0.6 μg/mL of ethidium bromide. Electrophoresis was carried out in TAE buffer. Ethidium bromide fluorescence was detected using a gel documentation system (Uvidoc, Bridgeville, UK).

2.5. AsPC-1 transfection

AsPC-1 cells were seeded on 24-well plate at a seeding density of 100,000 cells/well and cultured at 37 °C in a humidified atmosphere of 5% CO2 and 95% air. The cell medium was RPMI 1640 (Gibco® Invitrogen Ltd., UK) supplemented with 10% fetal bovine serum (FBS). The cells were used at 70% confluency, 24 h post-seeding. Prior to transfection, culture medium was removed and the cells were rinsed with phosphate-buffered saline (PBS, pH 7.4). Cells were incubated with DEMC/pEGFP complexes and plasmid alone at 37 °C. After 5 h the formulations were removed, the cells were rinsed with PBS and grown in culture medium for 24 h to allow GFP expression. After 24 h paraformaldehyde (4%) was added to the cells for 20 min then the cells were washed with PBS and were exposed to Tritonx-100 (4%). After PBS wash, cells were stained with DAPI (4,6-diamidino-2-phenylindole), to visualize nuclei, for 2 min and again washed with PBS, then visualized by florescence microscopy. Also cells were incubated with Arrest-in (commercial reagent) at 37 °C and detected according to its protocol.

2.6. Flow cytometry

Cells exposed to transfection agents were trypsinized (trypsin 0.25%) and once detached, complete medium was added to inhibit trypsin. Cell suspensions were then transferred to flow cytometry tubes and GFP expression in the transfected cells was determined using a flow cytometer. For each sample, 5000–10,000 events were collected. Signals were amplified in logarithmic mode for fluorescence and WinMDI software was used to determine the GFP positive events. The numbers of negative and positive cells were displayed on a histogram.

2.7. Evaluation of cytotoxicity

Cells were seeded at 1×10^4 cells/well in a 96-well plate and grown overnight. Immediately prior to incubation with chitosan derivative, growth medium was removed. Polyplexes were applied on the cells at different N/P ratios. Incubations were made for 5 h before removal of media containing derivatives and replacement with cell culture medium. After 24 h, MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide, a yellow tetrazole, which is reduced to purple formazan in living cells) was added to each well and incubated for 3 h under normal growing conditions. At this point all media was removed and DMSO (dimethyl sulfoxide) was added to dissolve the insoluble purple formazan product into a colored solution. Plates were incubated for 30 min at 37 °C. Then absorbance of the colored solution was measured at 570 nm using an Eppendorf Biophotometer (Hamburg, Germany). The viability of the cells was calculated in comparison to untreated cells.

2.8. Lagrange's interpolation polynomial

As stated in the introduction, interpolations polynomial approximates the functions. In numerical analysis, a Lagrange polynomial

Table 1
Particle size and zeta potential of DEMC/pEGFP complexes.

N/P ratio	Size (nm) ± SD	Zeta potential (mV) ± SD
5	114.24 ± 93.95	+6.14 ± 1.71
10	217.04 ± 84.43	+8.81 ± 0.56
20	344.4 ± 25.3	+10.03 ± 1.26
40	570.4 ± 126.70	+16.45 ± 4.25

is the interpolating polynomial for a given set of data points in the Lagrange form. If the charge ratio, transfection and cytotoxicity data are given in $k+1$ points then the interpolation formula of Lagrange can be described as follows (Berrut and Trefethen, 2004):

Given a set of $k+1$ data points

$$(x_0, y_0), \ldots, (x_j, y_j), \ldots, (x_k, y_k)$$

where no two x_j are the same, the interpolation polynomial in the Lagrange form is a linear combination of Lagrange basis polynomials:

$$L(x) := \sum_{j=0}^{k} y_j \ell_j(x)$$

$$\ell_j(x) := \prod_{0 \le m \le km \ne j} \frac{(x - x_m)}{(x_j - x_m)}$$

$$= \frac{(x - x_0)}{(x_j - x_0)} \cdots \frac{(x - x_{j-1})}{(x_j - x_{j-1})} \frac{(x - x_{j+1})}{(x_j - x_{j+1})} \cdots \frac{(x - x_k)}{(x_j - x_k)}$$

The above formula is used to correlate between charge ratio and biological properties of the DEMC/pEGFP complexes towards AsPC-1 cells. It could also be used for any kind of vector and cell. In the above function, y_j are the transfection/cytotoxicity (dependent variables) data and x is the charge (N/P) ratio (independent variable) of the complexes. Where x is our input and y is the predicted output of the biologic system. The equations and plots are estimated with MATLAB software.

3. Results and discussion

3.1. Size and ζ-potential

The following results and discussions can lead us to observe the efficacy of DEMC as a gene delivery vector to pancreatic cancer cells. Table 1 demonstrates size and zeta potentials for DEMC/plasmid complexes at different N/P ratios. The results represent that by increasing the N/P ratio, positive charge of polyplexes also increases which is necessary for cell transfection and gene delivery.

As demonstrated in Fig. 1, the polyplexes stability was examined via gel electrophoresis, which is the motion of dispersed particles relative to a fluid under the influence of a spatially uniform electric field. In the case of nucleic acids, the direction of migration, from negative to positive electrodes, is due to the naturally occurring negative charge carried by their sugar-phosphate backbone. Thus, if the positive charged polymer, DEMC, forms complexes with plasmid, the nucleic acid will not move from negative to positive electrodes due to the positive charged complexes. In the control section the plasmid is placed in the well and because of its negative charge it has moved through the gel. Therefore, due to the polyplexes' positive charges, no movement is seen through the gel and they remain in the well. In conclusion, gel electrophoresis analysis experiments showed that N/P ratios higher than 5 were necessary in order to stabilize and retard the plasmid content of DEMC/plasmid self-assembled nanoparticles.

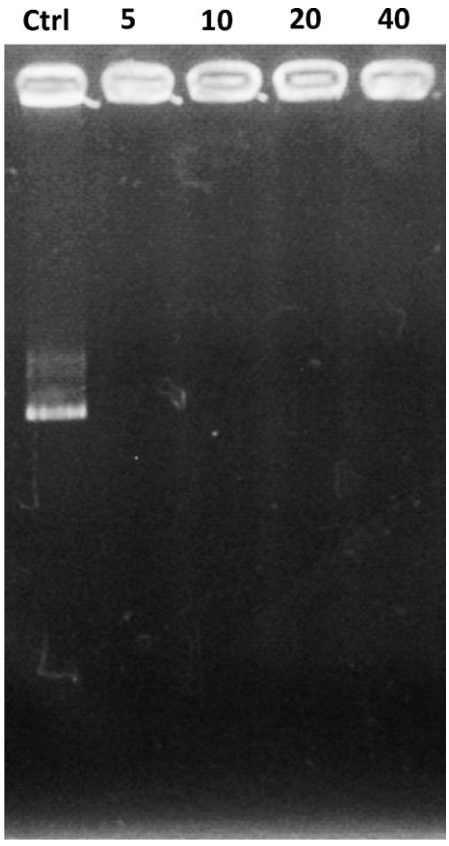

Fig. 1. Stability compare between different N/P ratios. Uncomplexed plasmid DNA is shown as control.

3.2. Transfection results

After characterization of polyplexes size, zeta potential and stability, DEMC/pEGFP complexes were used for transfection of pancreatic cancer cells in vitro. The transfection efficiency evaluated using fluorescent microscope and flow cytometry was dependent on the N/P ratio of the carrier to DNA. In Fig. 2 the fluorescence microscopy images of AsPC-1 cells transfected with different N/P ratios of DEMC/pEGFP and plasmid alone are demonstrated. In Fig. 3 the frequency histograms and dot plots are shown. Frequency histograms display relative fluorescence (FL1) plotted against the number of cells and dot plots represent the forward-scattered light signals (FSC) which is proportional to cell-surface area or size versus FL1.

According to Fig. 2, at charge ratios higher than 10, the number of GFP positive cells increases and reaches maximum at charge ratio of 40. For the plasmid delivery alone (negative control) no transfection is seen and few cells are transfected with Arrest-in, which because of its polymeric formulation it is used as a positive control (Fig. 2F and G). As it can be shown, the numbers of GFP positive cells transfected with complexes were higher as compared to a commercial transfecting reagent.

The quantitative analysis of Fig. 2 is seen in Fig. 3. As seen in Fig. 3A the percent of positive cells (cells with GFP) represents that increase in N/P ratio from 5 to 10, 20 and 40 results up to 5.28, 14.43 and 16.70-fold increase in transfection efficiency, respectively.

To show the changes in AsPC-1 cells after transfection with different charge ratios of DEMC/pEGFP, the results in Fig. 3A were also analyzed by charts of cell size and mean fluorescence intensity (MFI) against N/P ratio. A comparison of MFI in the transfected cells, relative to control, shows 120 ± 9.28%, 325 ± 42.68%,

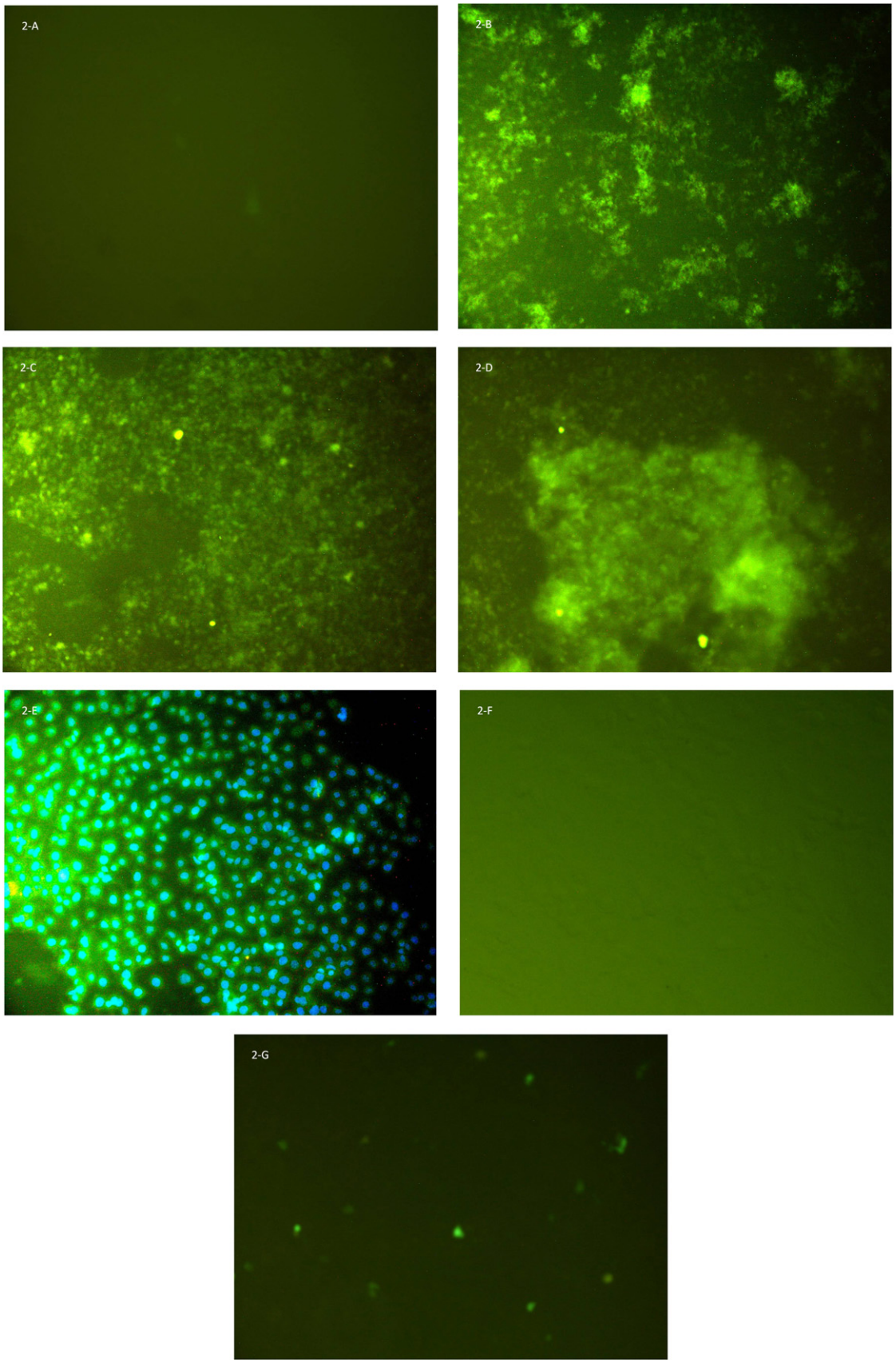

Fig. 2. Cell transfection results. A-D) Fluorescent microscopy of cells transfected with DEMC/pEGFP complexes at different N/P ratios, (A) N/P = 5, (B) N/P = 10, (C) N/P = 20, (D) N/P = 40, (E) Transfected cells with DAPI colored nuclei (blue), at higher magnification. (F) Cells transfected with plasmid alone. (G) Cells transfected with Arrest-In (commercial transfecting reagent). (For interpretation of the references to color in this figure legend, the reader is referred to the web version of the article.)

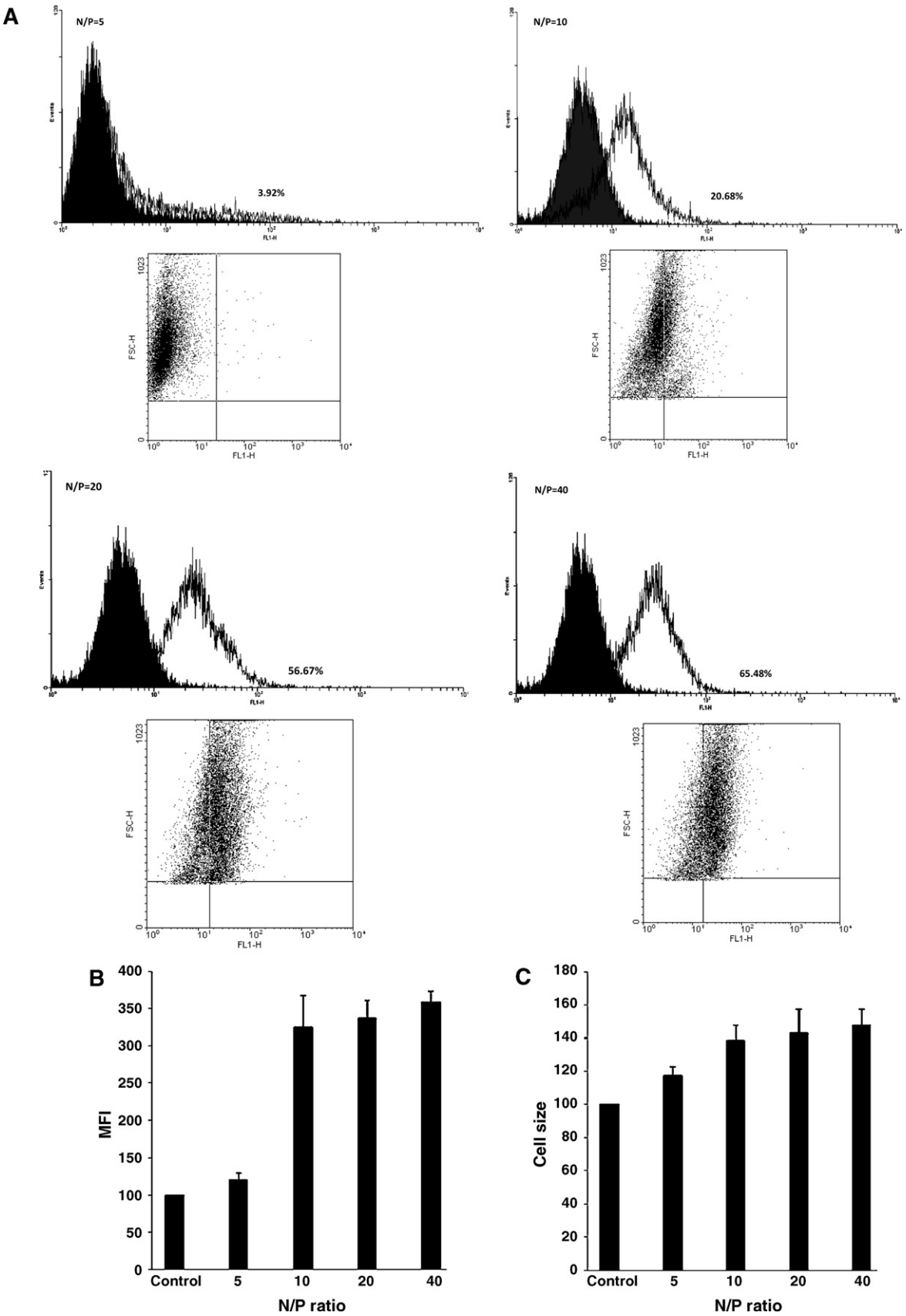

Fig. 3. (A) Flow cytometry histograms and dot plots of polyplex's cellular uptake. The black histograms are untreated cells (control) and the white histograms are treated cells with DEMC/pEGFP complexes. The dot plots represent FSC (Forward-scattered) versus relative fluorescence (FL-1). (B) Quantitative analysis of MFI (mean fluorescence intensity) versus N/P ratio which is given in percentage relative to the control, set as 100% (±standard deviations). The given numbers represent the average. C) Quantitative analysis of the mean cell size. Cell size is given in percentage relative to the control, set as 100% (±standard deviations). The given numbers represent the average.

337.24 ± 23.71% and 359 ± 14.60% increase in fluorescence intensity at N/P ratios of 5, 10, 20 and 40, respectively (Fig. 3 B).

Also after transfection, cell sizes have increased 117.22 ± 5.57%, 138.55 ± 9.24%, 142.88 ± 14.39% and 147.91 ± 9.37% by increase in charge ratio (Fig. 3C). Considering the fact that all the datas gained from flow cytometry are relative to a negative control and there is no absolute number, so MFI and Cell size are given in percentage relative to the control, set as 100% (±standard deviations) and the given numbers represent the average.

This effect can be explained by the fact that, when a cationic polymer is used at an excess ratio of cationic charges to nucleic acid phosphates, the resulting nucleo-polymer particles will fix to the cell surface. Electrostatic interactions between the positively charged DEMC/pDNA complexes and anionic proteoglycans of the cell membranes will lead to endocytosis. This shows that by increasing in charge ratio, zeta potential increases and so because the cells have up taken more polyplexes, their size has also increased.

According to the searches done by McNaughton et al. (2009), even sizes of μm can be transfected at high zeta potentials. Thus the positivity of the nanoparticles is important for their interaction with the cellular membrane components (Table 1). Just like other non-viral vectors, transfection efficiency of cationic DEMC depends on the vector characteristics like chemical structure of the vector, particle charge and size, N/P ratio, and moreover on the type of cell used for transfection targeting.

Chemical modification of vectors, e.g. N-alkylation and quaternization, are expected to increase transfection efficiency by modulating complex interactions with cells, such as adsorption on cell surfaces and cell uptake. Moreover, hydrophobic units in the polymeric carriers may assist dissociation of polymer/DNA complexes, to facilitate release of DNA which otherwise would be strongly bound through ionic interactions between cationic units and phosphates of DNA. Thus in DEMC, these favorable characteristics of the hydrophobic units, two ethyl groups, has lead to high transfection efficiency.

About particle charge and size, a positive surface charge allows better uptake of the DEMC nanoparticles across the cell membrane. Also the mathematical models suggested that the rate of cellular uptake of both particles was fast in apoptotic and necrotic tissues. Furthermore, the uptake kinetics of positive particles is irreversible, but is reversible for negative particles over the timescales investigated (Kim et al., 2010). Particle size is also an important factor in particulate delivery systems. It is closely associated with the targeted lymphatic system and cell uptake.

In constructing gene complex carriers, the N/P ratio may play an important role in influencing the degree of complexation particle diameter, transfection efficiency, and cytotoxicity of carriers.

In addition to complex formation with DNA, an efficient gene delivery system is required to transport the gene into the cell and see to its eventual release, leading to gene expression and subsequent protein synthesis. Since chitosan-mediated transfection depends on the cell type, it is thus necessary to test a gene carrier on different cell lines, especially cells that resemble those that will be targeted. Cellular membrane composition varies among cellular types and may facilitate or hinder the binding of the complexes and subsequent internalization (Von Gersdorff et al., 2006).

Also as seen in Figs. 2 and 3 there is a sudden increase in transfection from N/P ratio of 5–10. This means that at Lower charge ratios, DEMC/plasmid complexes cannot affect the cell surface and the particles, although attached, were not able to effectively enter the cells. It is obvious from Fig. 3B that an excess of cationic polymer is necessary to cause the uptake of nanoparticles. Obviously, higher charge ratios (Table 1) have more impact on the cell membrane, because the probability of the interaction of the polymer with the membrane will increase and this is the reason of sudden increase in transfection from charge ratio of 20–40. Another explanation for this observation could be the effect of a higher charge ratio causing higher affinity to negatively charged cell surfaces. These negatively charged cell surfaces include MUC-1 mucins, which are over expressed in Aspc-1 cell line (Ohuchida et al., 2006). This affinity can induce more endocytosis of the particles. The mechanism of uptake needs to be clarified, but it is possible that the hole formation may be the main mechanism, since nanoparticles at the lower charge ratio (N/P = 5) had very low cell uptake and transfection (Fig. 2A). For large particles of DEMC/plasmid, other mechanisms of uptake, such as macropinocytosis, should be considered in further investigations as well.

In comparison with other researchers' studies, the transfection results with other quaternized chitosan derivatives, Kean et al. (2005) proposed that TMC increases gene–polymer interaction and its transfection efficiency. Also Thanou et al. (2002) prepared trimethlyated chitosan oligomers and investigated their transfection efficiency. The results indicated that TMC/DNA complexes appeared to be superior to oligomeric chitosan transfection in COS-1 (monkey kidney fibroblasts) cell line. In a study done by Germershaus et al. (2008), chitosan, trimethyl chitosan and polyethylenglycol-graft-trimethyl chitosan/DNA complexes were characterized and the result showed that quaternization of chitosan strongly reduces aggregation tendency and pH dependency of DNA complexation. Accordingly, cellular uptake was increased 8.5-fold compared to chitosan polyplexes resulting in up to 678-fold increased transfection efficiency in NIH/3T3 (mouse embryo) cells. In accordance with researchers' reports, it was found that transfection efficiency of TMC and PEG-TMCs increased with increasing N/P ratio and charge ratios between 10 and 30 were found to result in significant transfection of NIH/3T3 cells.

As discussed above, in our study, charge ratios between 10 and 40 of DEMC/plasmid complexes have significant transfection to AsPC-1 cells. Thus, these charge ratios could be used for further studies on DEMC as a non-viral vector.

3.3. Cytotoxicity

In order to investigate the cytotoxicity of the chitosan derivative on AsPC-1 cell line, the MTT assay was used. Derivatives were applied to the cells at concentrations ranging from 2×10^4 to 5×10^3 μg/ml for 5 then 24 h and the effect on cell viability was measured. Viability data are shown in Fig. 4. It shows that, toxicity of the nanoparticles is dependent on N/P ratio.

According to researchers' studies on cytotoxicity with other quaternized chitosans, TMC showed a general trend of increasing toxicity with increasing degree of trimethylation. However, higher toxicity was seen in polymeric chitosan derivatives over oligomeric chitosan derivatives at similar degrees of trimethylation (Thanou et al., 2002).

In this study, the cells treated with plasmid alone remained 100% viable. At N/P ratios of 20 and 40, cells treated with DEMC polymer alone had a viability of 37 and 28 percent. DEMC/pEGFP complexes induced a decrease in viability of AsPC-1 cell line to 34 and 28 percent for N/P ratios of 20 and 40, respectively. The least cell toxicity is for N/P = 10, which 60 and 55 percent of the cells remain viable after exposure to DEMC alone and DEMC/pEGFP complexes.

Increase in cytotoxicity of this polymer can be explained by the fact that the relatively large polyplexes induce cell death probably due to the more efficient uptake. Since by increasing the N/P ratio, positive charge also increases so uptake of polyplexes and also unbound DEMC or a more intimate interaction and disruption of the plasma membrane results in higher toxicity. As seen in Fig. 4, after transfection the cell size increases and since increase in cell size is associated with cell death; it suggests that the transfection procedure induces cell death at higher charge ratios. This is due

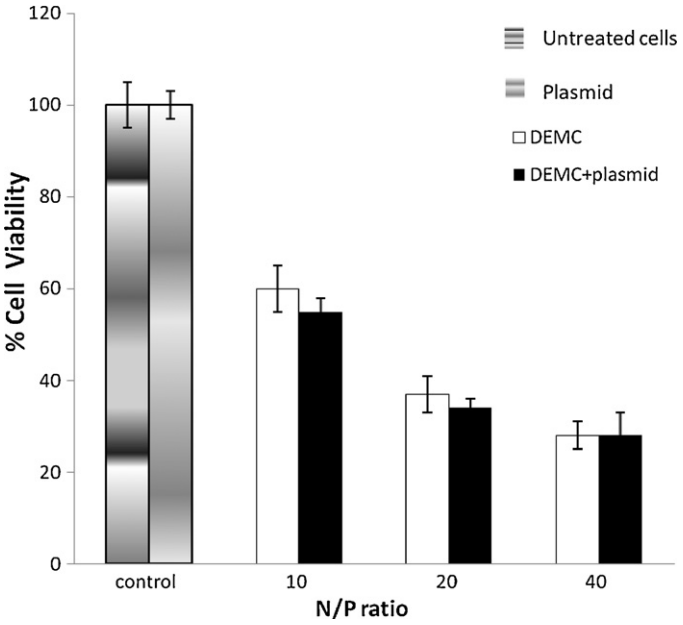

Fig. 4. Cell viability after treatment of Aspc-1 cells with DEMC polyplexes.

to the increase in particles size, which results an increase in cell size and ultimately cell death. Increase in positivity of the particles is the other reason for the observed cytotoxicity of DEMC/pEGFP complexes. Also the hydropobic units on the polymer can increase cell interaction and damage.

3.4. Interpolation polynomial functions

The equations and plots estimated with MATLAB software are stated in Eqs. (2) and (3) and displayed in Fig. 5. According to Eq. (1), at different charge ratios, the percent of transfected and viable cells exposed to DEMC/pEGFP polyplexes can be predicted.

$$y = \sum_{i=0}^{n} \frac{(x - x_0)\ldots(x - x_{i-1})(x - x_{i-1})\ldots(x - x_n)}{(x_i - x_0)\ldots(x_i - x_{i-1})(x_i - x_{i+1})\ldots(x_i - x_n)} \quad (1)$$

By replacing our transfection and cytotoxicity datas (y) at different N/P ratios (x), 5–40, in the above equation, the following results are obtained.

$$\text{Transfected cells}(\%) = 4.28 \times 10^{-4} \times 10^{-2}x^3 + 0.88x^2 - 4.51x + 8.51 \quad (2)$$

$$\text{Viable cells}(\%) = -3 \times 10^{-3}x^3 + 27 \times 10^{-2}x^2 - 8.1x + 112 \quad (3)$$

As seen in Fig. 5, plot A and B, it is predicted that at charge ratios of 5–25, transfection increases and cell viability decreases. At N/P ratios between 25 and 35 transfection decreases and cell viability shows a steady state situation. At N/P = 35 transfection of the AsPC-1 cells increases and reaches maximum at charge ratio of 40. At this range (35–40), cell viability will decrease. Apparently, for nanoparticles internalized into cells by endocytosis, an increase in N/P ratio will increase polyplexes positive charge (Table 1), which can promote cell damage and reduce viability.

It is clear that these are only predictions and considering the highly nonlinear nature of most biological systems (Bewick et al., 2009), more studies on route of uptake and intracellular processing are necessary to fully understand the gene delivery process via DEMC/plasmid complexes to AsPC-1 cells and explain the predicted results of presented mathematical model.

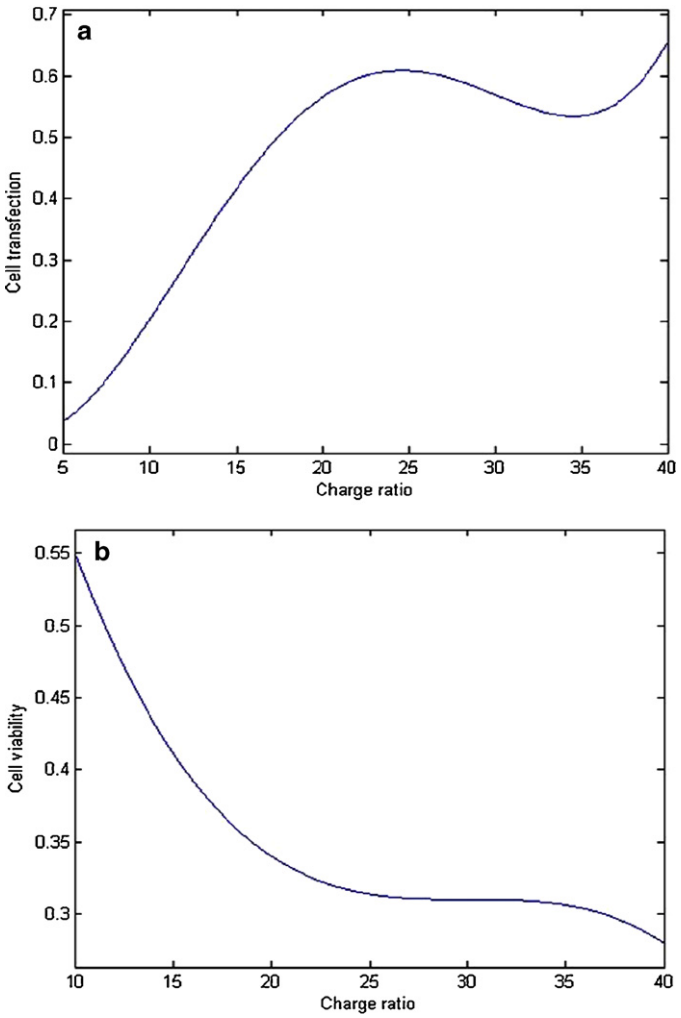

Fig. 5. The relation between charge ratio, cell transfection (A) and cell viability (B) predicted with Lagrange's interpolation polynomial method.

4. Conclusion

In summary, in this study a bio-based material is introduced which it could be used for drug delivery and nanomedicine. Quaternized modifications of chitosan present characteristics that are useful in DNA condensing and efficient gene delivery. DEMC was able to condense DNA and transfect AsPC-1 cells. A mathematical model is proposed for better understanding of its function. According to these studies, the route for efficient transfection of nanomedicines may depend both on the cell line and polyplexes (Goncalves et al., 2004; Rejman et al., 2005; Ishii et al., 2001). So it is hypothesized that mathematical models could be used for relating polyplex's nonbiologic and biologic properties and they might be specific for each delivery vector and cell line used. This model may be used to conduct experiments, which are too difficult or costly to carry out directly. The biological and the mathematical results both showed that the increase of cell viability is correlated with the decrease of N/P ratio in polyplexes and charge ratio of 10, due to its highest cell viability, can be chosen for further research in gene delivery to pancreatic cancer cells. Therefore, more research activities are planned to use this charge ratio for pancreatic cancer gene delivery in vivo and the results will be considered for publication soon.

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