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Inhibition of EGFR expression with chitosan/alginate nanoparticles encapsulating antisense oligonucleotides in T47D cell line using RT-PCR and immunocytochemistry

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ABSTRACT

Chitosan/alginate nanoparticles optimized for size and loading efficiency were evaluated for their potential of antisense oligonucleotide delivery. The antisense for epidermal growth factor receptor (EGFR) that is over-expressed in many cancer cells was loaded in chitosan/alginate nanoparticles. The T47D breast cancer cell line was chosen to study the efficiency of optimized nanoparticles (chitosan/alginate 1:1, alginate/calcium chloride 0.2% and N/P ratio of 5 and 25) on EGFR expression. The MTT cytotoxicity evaluation of nanoparticles confirmed non-toxic properties of these carriers. The FITC-labeled EGFR antisense showed that T47D cells can uptake antisense-loaded nanoparticles better than naked antisense. Both RT-PCR and immunocytochemistry analyses showed that nanoparticles with N/P ratio of 5 can down-regulate the expression of EGFR in T47D breast cancer cell line by improving internalization and stability of antisense molecules.

1. Introduction

Gene therapy of disorders by plasmid DNA to restore gene expression or inhibition of gene expression by antisense oligonucleotides or siRNA is promising approach for the treatment of diseases such as cancers in future. The main obstacle for therapeutic application of antisense or siRNA is the lack of a safe and efficient delivery system for nucleotide-based drugs in vivo (Taetz et al., 2009).

Generally, two different approaches have been utilized for the delivery of nucleic acids in gene therapy including viral and non-viral vectors (i.e. cationic polymers or lipids). Although viral vectors show excellent transfection efficiencies but they develop a high immunogenicity after repeated administration. In addition, viral vectors have potential oncogenicity due to insertional mutagenesis and they have limitation to carry large size of DNA. Another problem with viral vectors is transfection of specific cell types or tissues via inclusion of a targeting moiety (Merdan, Kopecek, & Kissel, 2002).

Non-viral systems, especially polymers show significantly lower safety risks and are capable of carrying DNA molecules. They can be produced in large quantities easily and inexpensively. The major disadvantage of them is their toxicity and low transfection efficiency (Merdan et al., 2002).

Requirements for an ideal drug delivery system are biocompatibility, biodegradability and low antigenicity, protection of the drug, maintenance of the integrity till the target is reached, avoidance of side effects, membrane passage, target recognition and association, controlled drug release, and elimination upon drug release (Ciofani, Raffa, Menciassi, & Dario, 2008).

Considering to aforementioned requirements among different polymers, chitosan/alginate nanoparticles were chosen in our study because they are natural polymers which are widely used in gene delivery.

Chitosan is a cationic natural biopolymer produced by alkaline *N*-deacetylation of chitin, the most abundant natural polymer after cellulose and has good biocompatibility, biodegradability, low toxicity and low cost. It has been used in gene delivery systems to enhance the transfection efficiency and stability of loaded molecules,

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and to reduce cytotoxicity of the delivery vector (Kim & Kim, 2007).

To improve the transfection efficiency of chitosan, it is suggested that a secondary anionic polymer can reduce the strength of interaction between chitosan and gene or oligonucleotide. Uses of some anionic polymers like PLL and PEI which are toxic were reported before (Ciofani et al., 2008). Alginate is chosen in this study which is a non-toxic biocompatible anionic biopolymer. Alginate is a co-polymer extracted from some types of brown algae and it is made up of two uronic acids: D-mannuronic acid and L-guluronic acid. The reticulation process consists of the simple substitution of sodium ions with calcium ions. The relatively mild gelation process has enabled not only proteins, but also cells and DNA to be incorporated into alginate matrices with retention of full biological activity (Ciofani et al., 2008).

Whereas the chitosan/alginate (Chi/Alg) system has been widely studied at the micro- and macro-scales for drug delivery, its development at the nano scale for gene delivery has been limited. We previously reported the development of chitosan/alginate nanoparticles through a parametric study designed to optimize preparation conditions. Optimization of particle size and loading efficiency as the responses were carried out by Box–Behnken response surface methodology, and optimized formulation was reported as nanoparticles with chitosan/alginate ratio 1:1, alginate/calcium chloride 0.2% and N/P ratio of 5 (molar ratio of amine group of chitosan to phosphate group of antisense) (Gazori et al., 2009). The present study investigates the suitability of chitosan/alginate nanoparticles as biocompatible, non-toxic, and non-viral vectors. The epidermal growth factor antisense (EGFR AS) loading in chitosan/alginate nanoparticles was evaluated for transfection efficiency and cytotoxicity using T47D cells. This cell line was selected because EGFR is over-expressed in it, and EGFR over-expression is associated with a poor prognosis in breast carcinoma, often revealing advanced disease (Valdehita et al., 2009).

EGFR is a 170-kDa transmembrane protein that upon binding to its ligand, the ligand–receptor complex undergoes dimerization and internalization. Activation of the intracellular protein kinase via autophosphorylation leads to signal transduction and consequent cellular functions (Kim, Khuri, & Herbst, 2001).

Although EGFR is important in the maintenance of normal cellular function and survival, EGFR expression clearly appears to contribute to the growth and survival of tumor cells. The EGFR signal transduction pathways have therefore been correlated with various processes that contribute to the development of malignancies, such as effects on cell cycle progression, inhibition of apoptosis, angiogenesis, tumor cell motility, and metastasis. Because of the importance and the role of the EGFR pathways in cell cycle progression and tumor cell proliferation, different strategies were developed to block or downregulate EGFR. These include monoclonal antibodies to the EGFR, tyrosine kinase inhibitors, ligand-linked toxins, and antisense approaches (Kim et al., 2001).

Antisense oligonucleotides are short lengths of single stranded RNA or DNA with base sequences complementary to a specific gene or its mRNA. They offer the exciting possibility of selectively modifying the expression of a particular gene without affecting the function of others. As such, antisense oligonucleotides are useful tools in the study of gene function and have also aroused interest as possible therapeutic agents. When targeted to mRNA or its precursor (pre-mRNA), antisense oligonucleotides hybridize to the target sequences by Watson–Crick base pairing. The subsequent formation of the RNA–oligonucleotide duplex then prevents the translation of the message into the encoded protein. Such a strategy may be useful in activating tumor suppressor genes in the treatment of cancer (Akhtar & Juliano, 1992).

Due to importance of inhibition of gene expression in new cancer therapeutic approaches and lack of efficient antisense or siRNA delivery systems, in this study the alginate/chitosan nanoparticles were evaluated to deliver EGFR antisense to human breast cancer T47D cells for down regulation of EGFR expression.

2. Materials and methods

2.1. Materials

Sodium alginate (BDH Co., UK), low molecular weight chitosan (Sigma–Aldrich Co., Germany), calcium chloride (Merck KGaA Co., Germany), EGFR phosphorothioated 21 mer antisense 5′ (TTT CTT TTC CTC CAG AGC CCG) 3′ (TIB MOL BIOLP®, Germany), RPMI1640 (Biosera, UK), heat-inactivated fetal bovine serum (Biosera, UK), penicillin and streptomycin (Biosera, UK), MTT dye (3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide, Sigma–Aldrich Co., Germany) were purchased and used as received.

2.2. Particle preparation

With respect to chitosan/alginate ratio a certain amount of sodium alginate stock solution was diluted with 10 ml of filtered deionized water. Then 1 ml of calcium chloride solution adjusted to alginate/calcium chloride percentage ratio was added dropwise to aforementioned solution while stirring. The prepared alginate/calcium chloride pregel was stirred for a further 10 min. Chitosan solutions were prepared according to N/P ratios of 5, 15 and 25 and different chitosan/alginate ratios of 0.5, 0.75 and 1. Then 5 μl of 100 μM of EGFR antisense solution was added to it. It was stirred for 10 min to be complexed, and then added dropwise to calcium alginate pregel, while stirring. In cases where the pregel had large aggregates these were broken up using bath sonicator. The pH of the solution was adjusted to 5.3 using 0.1 N NaOH solution, and was stirred for further 30 min. All samples were centrifuged at 1100 rpm for 15 min to remove any large aggregates prior to analysis. Centrifugation under these conditions allowed aggregates to form pellet, leaving nanoparticles suspended in the supernatant. The particle suspension was then centrifuged at 25 °C in the Amicon® Ultra-15 (Ultracel-100K) centrifuge tube with 100 kDa cut-off at 5000 rpm for 20 min to separate free polymers from nanoparticles. Nanoparticles in the dialysis tube were evaluated for their size and zeta potential. Then the absorbance of the solutions in the tubes was measured at 260 nm using a UV/VIS spectrophotometer (Jasco V-530, Jasco Co., Japan). The amount of AS ODN associated with the nanoparticles was calculated by the difference between the initial amount of AS ODN added to the chitosan and the amount measured in the supernatant.

According to our studies (Gazori et al., 2009), the optimum conditions for preparation of chitosan/alginate nanoparticles with the smallest size (194 nm) and best loading efficiency (95.6%) was chitosan/alginate ratio of 1, alginate/calcium chloride ratio of 0.2% and N/P ratio of 5 at pH 5.3 using Box–Behnken response surface methodology. A second formulation which had chitosan/alginate ratio 1, alginate/calcium chloride 0.2% and N/P ratio of 25 with particle size of 430 nm and loading efficacy of 66.5% was also chosen. This formulation with N/P ratio of 25 was used because in this study we intend to check the prepared nanoparticles in cell culture at least in two different N/P ratios to evaluate the effect of N/P ratio on transfection efficiency.

2.3. Cell culture

The human breast cancer T47D cell line (ATCC, HTB-133) was obtained from Pasteur Institute Cell Bank of Iran (Tehran, Iran). T47D cells were grown in RPMI1640, containing 10% heat-

inactivated fetal bovine serum, 100 U/ml penicillin, and 100 ng/ml streptomycin at 37 °C in humidified air containing 5% CO_2.

2.4. MTT cytotoxicity assay

To evaluate the number of live and dead cells, the cells were stained with trypan blue and counted using hemocytometer. To determine the growth inhibitory activity of the test compounds, 1×10^4 cells were plated into each well of 96-well plates in 180 μl of growth medium. After 48 h of seeding, the cells in each set of three wells were treated with doxorubicin (DOX) 250 nM and EGFR antisense alone (with two different concentrations 500 and 1500 nM), alginate and chitosan solution (50 μg/ml), alginate and chitosan with two different concentrations of antisense (500, 1500 nM) for 4 h. In another experiment doxorubicin 250 nM, EGFR AS 500 nM, alginate and chitosan solutions (50 μg/ml), chitosan/alginate nanoparticles without antisense, and optimized chitosan/alginate nanoparticles with N/P ratios of 5 and 25 were tested in each set of three wells for 4 and 24 h of incubation time. Control wells were treated only with culture medium. After exposure times; in both experiments, all the treatments were changed with RPMI and the plates were incubated for 48 h at 37 °C and 5% CO_2. The growth rate was determined using the MTT assay as described previously (Bazargan et al., 2008). Briefly, 25 μl of MTT (4 mg/ml in PBS) was added to each well and incubated for 3 h at 37 °C. The medium and MTT were then removed from the wells, and formazan crystals were dissolved in 100 μl of DMSO. The absorbance was recorded in a microplate reader (Sunrise Tecan, Austria) at a test wavelength of 540 nm and a reference wavelength of 690 nm. Each experiment was repeated three times each in triplicate format, and results were expressed as means ± SE.

2.5. Cell uptake of the nanoparticles

Cellular uptake of EGFR antisense (AS) nanoparticles were investigated using 5′ end FITC-labeled EGFR AS alone and chitosan/alginate nanoparticles with two different N/P ratios were produced in dark place. Then 1000 μl of formulations in FBS free RPMI were added to each well of six-well plate whose medium was removed and washed with PBS previously. The cells were incubated for 4 h at 37 °C and 5% CO_2 at dark. Then the medium of each well was removed and washed by PBS. Then wells with each treatment were evaluated by inverted fluorescent microscope (Olympus IX51, Olympus Inc.) at 100× and 400× magnifications.

2.6. RNA isolation

T47D cells were seeded in six-well plates in RPMI1640 culture medium and incubated in humidified CO_2 incubator (5% CO_2, 37 °C). After 48 h culture medium was changed and RPMI was added to negative control and doxorubicin, EGFR AS, nanoparticles without AS and nanoparticles containing AS with two different N/P ratios were added to the other wells. All the treatments were prepared in FBS free RPMI because of the probability of reaction between the FBS contents and polymers in the nanoparticles. Then the solutions were removed after 4 h and RPMI was added to all wells. After 48 h total RNA was isolated using Tripure reagent (Roche, Germany) according to the method previously described (Kaabinejadian, Fouladdel, Ramezani, & Azizi, 2008).

2.7. RT-PCR

The cDNAs were synthesized from 2 μg total RNA by incubation for 1 h at 42 °C with M-MLV reverse transcriptase (Fermentas, EU) and oligo$(dt)_{18}$ primer according to the manufacturer's instruction. Then 2.5 μl of the reaction mixture was subjected to polymerase chain reaction (PCR) to amplify the sequence of EGFR using specific primers (forward 5′-CAACATCTCCgAAAgCCA; reverse 5′-CggAA CTTTggg CgA CTAT), (TIB MOLBIOL, Germany) for EGFR. As an internal control, the house keeping gene β-actin (forward 5′-gTCC TgTggCATCCACgAAACT; reverse 5′-TACTTgCgCTCAggAggAgCAA) was co-amplified in each reaction. The PCRs were carried out in final volumes of 50 μl containing 1.5 mM $MgCl_2$, 0.2 mM dNTP, 0.4 μM of each oligonucleotide primer and 2.5 U of *Taq* DNA polymerase. PCR condition for β-actin amplification was 35 cycles of 95 °C for 30 s, 57 °C annealing for 30 s, and 72 °C extension for 45 s, and for EGFR amplification was 35 cycles of 95 °C for 15 s, 57 °C annealing for 15 s, and 72 °C extension for 30 s. The PCR products were visualized using 1.5% agarose gel electrophoresis with ethidium bromide staining under UV illumination (Bio Doc-IT System, USA). In negative control cDNA was replaced by DEPC (diethylpyrocarbonate) treated water (Kaabinejadian et al., 2008).

2.8. Immunocytochemical analysis of EGFR expression

The T47D cells (5×10^4) were seeded in eight-well chamber slides (Lab Teck, USA) in RPMI1640 culture medium and incubated in a humidified CO_2 incubator (5% CO_2, 37 °C). After 48 h culture medium was removed and RPMI was added to negative control chamber slide, and doxorubicin, EGFR AS, nanoparticles without AS and nanoparticles containing AS with two different N/P ratios were added to the other chamber slides. All of the treatments were prepared in FBS free RPMI. After 4 h treatments were changed with RPMI1640 and remained for 48 h. Then cells were washed with PBS and fixed in methanol:acetone (9:1) at −20 °C. Endogenous peroxidase activity and non-specific binding sites were blocked by incubating cells in H_2O_2/methanol and BSA 3% for 30 min each, respectively. Cells were then incubated overnight at 4 °C with EGFR antibody. The primary antibody for EGFR (Cell Signaling, Switzerland) was used at dilution of 1:100. The results were visualized using the streptavidine–biotin immunoperoxidase detection kit (LabVision, USA) and DAB chromogen (DakoCytomation, Denmark) based on manufacturer's instruction with necessary modifications. Finally, cells were counterstained with Meyer's hematoxyline (DakoCytomation, Denmark), mounted and studied under light microscope. Negative control cells were incubated with antibody diluent alone (Kaabinejadian et al., 2008).

2.9. Statistical analysis

For comparison of two means, Student *t*-test was applied and for more than two means, Kruskal–Wallis one way analysis of variance on ranks was selected with Student–Newman–Keuls method post hoc. A P value <0.05 was considered significant difference among treatments.

3. Results and discussion

3.1. Cytotoxicity assay

The MTT test was used to evaluate the effect of the polymers and nanoparticles on the metabolic activity of T47D cells. No evidence of cytotoxicity was observed for polymers or nanoparticles (Figs. 1 and 2). Moreover, the statistical analysis of the results showed the higher mean values of cell viability (% of control) in the groups treated with chitosan and alginate. Chitosan solution showed significant difference with control RPMI ($P < 0.05$). This is probably an indication that chitosan may influence lysosomal and mitochondrial activity of the cells (Borges et al., 2006). Ishii et al. reported that chitosan (10 kDa) improves the viability of L132 human embryonic lung cells and CCRF-CFM human

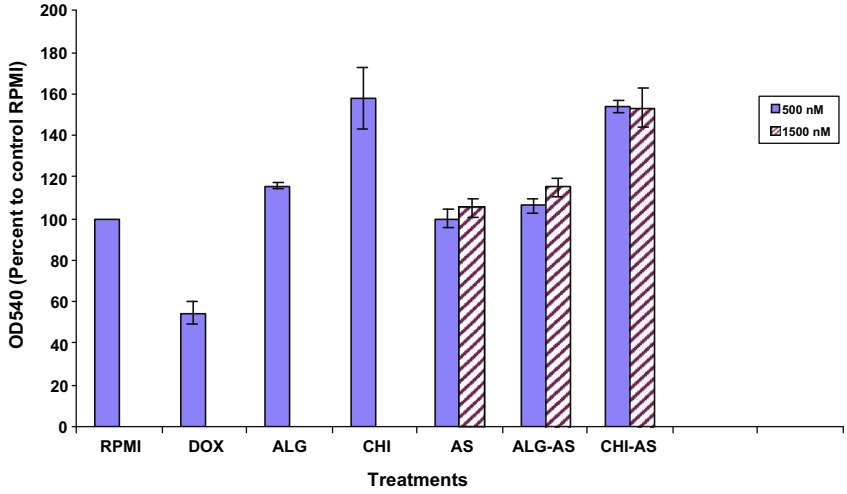

Fig. 1. Cytotoxicity of test compounds on T47D cells. The T47D cells were cultured in 96-well plates and exposed for 4 h to doxorubicin 250 nM, alginate and chitosan solution (50 μg/ml), EGFR antisense alone (at two different concentrations 500 and 1500 nM), alginate and chitosan with two different concentrations of antisense (500, 1500 nM) and negative control of RPMI as explained in Section 2. All data are presented as mean of three different measurements ± SE.

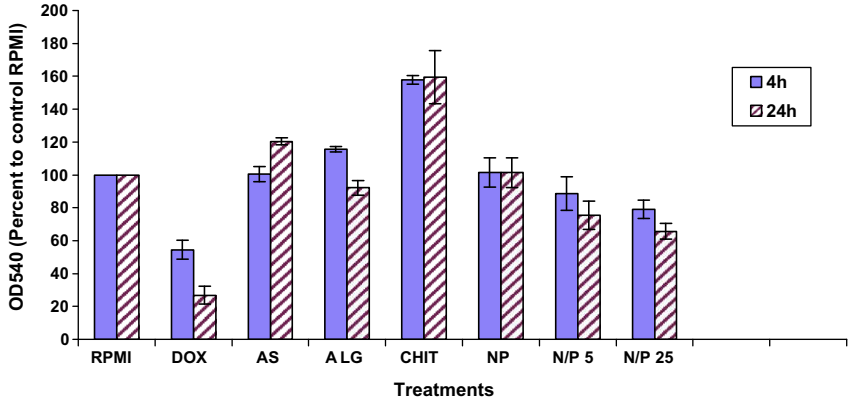

Fig. 2. Cytotoxicity of test compounds on T47D cells at different time-points. The T47D cells were cultured in 96-well plates and exposed to doxorubicin 250 nM, EGFR AS 500 nM, alginate and chitosan solution (50 μg/ml), chitosan/alginate nanoparticles without AS, optimized chitosan/alginate nanoparticles with N/P ratios of 5 and 25 for 4 (blue filled columns) and 24 (hatched columns) h of incubation time and negative control of RPMI. All data are presented as mean of three different measurements ± SE. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

lymphoblastic leukemia cells, compared to control cells at concentrations of 0.1–1000 μg/ml (Ishii, Okahata, & Toshinori Sato, 2001).

Douglas et al. (2006) also reported that chitosan/alginate nanoparticles showed no toxicity to 293T cells when incubated at low concentrations. At the lowest concentration tested, neither nanoparticle–DNA complexes nor unloaded nanoparticles did show difference from controls at 6 or 24 h. However, increasing the concentration of nanoparticles resulted in decreased viability over 6 and 24 h exposures, with the highest concentration demonstrating statistically reduced viability over the two lesser concentrations after 24 h (Douglas et al., 2006).

Concentrations of 500 and 1500 nM naked AS had no significant toxicity on T47D cells compared to the control RPMI. There was also no significant difference between the toxicities of these two concentrations of AS condensed in chitosan nanoparticles, but in alginate solution with AS there was significant difference between 500 and 1500 nM concentrations ($P < 0.05$) (Fig. 1).

It has also been reported that the best transfection efficiency is obtained with 500 nM AS in dendritic vehicle on A431 cell line (Hussain et al., 2004).

The duration of treatment time was evaluated in 4 and 24 h incubation times of cells with different treatments (Fig. 2). It was observed that there is significant difference between these two incubation times in cells treated with doxorubicin, naked AS, alginate solution and nanoparticles with N/P ratio of 25. But no significant difference was observed (Fig. 2) between treatment time in chitosan solution and nanoparticles with N/P ratio of 5 ($P < 0.05$). Therefore, 4 h treatment time was chosen to minimize the toxicity of the vehicle.

There was not any significant difference between RPMI (negative control) and chitosan/alginate nanoparticles without AS which approved that these nanoparticles are non-toxic carriers. There was significant difference between cytotoxicity of doxorubicin solution (250 nM) and all of the other treatments. Also there is significant difference between cytotoxicity of alginate solution and both nanoparticles (N/P ratio of 5 and 25). Proliferative effect of chitosan solution was significantly different with all of the other treatments ($P < 0.05$) (Fig. 2).

3.2. Cellular uptake

Previous studies have shown that in other cell lines (e.g. Caco-2) that the particle uptake depends on their size, with nanoparticles showing relatively greater efficiency of uptake than microparticles.

The uptake has been shown to depend on the temperature of incubation, with relatively lower uptake at 4 °C than at 37 °C, suggesting that the uptake is an energy dependent process. The uptake of nanoparticles seems to be mediated by endocytosis (Davda & Labhasetwar, 2002), which in turn depends on temperature, structure, concentration of AS and cell type. At relatively low AS concentration, internalization occurs via interaction with a membrane bound receptor, and AS molecules do not readily cross the cellular membrane because of their negative charge and size. Therefore, the simple addition of naked AS does not result in effective internalization (Fattal & Bochot, 2008).

The T47D cells which were treated with FITC-labeled EGFR AS are shown in Fig. 3. Obviously, Fig. 3A shows that naked FITC-AS were localized on the cell surface and negligible amounts of FITC-AS could enter to the cytoplasm, but nanoparticles could internalize AS molecules into the cells (Fig. 3B and C). Also this study showed that chitosan/alginate nanoparticles with N/P ratio of 5 can be uptaken more than N/P ratio of 25. It might be due to the fact that nanoparticles with N/P ratio of 5 are smaller than 25. It seems that particle size is a more important parameter for internalizing the cells than electric potential of nanoparticles surface.

3.3. RT-PCR

The transfection efficiency may depend on several factors such as chemical structure of polycations, size and composition of complexes, surface charge, interaction between cells and complexes and the cell type (Mansouri et al., 2004; Nafee, Taetz, Schneider, Schaefer, & Lehr, 2007). In addition Ishii et al. reported that the molecular mass of chitosan, plasmid concentration, stoichiometry of complex, serum concentration, and pH of the transfection medium can influence the transfection efficiency (Ishii et al., 2001).

Figs. 4 and 5 show that chitosan/alginate nanoparticles with N/P ratio of 5 can downregulate the expression of EGFR mRNA. There is significant difference between these nanoparticles with naked AS and also with nanoparticles with N/P ratio of 25, but there is no significant difference between naked AS and AS loaded nanoparticles with N/P ratio of 25. In addition, there is significant difference between 250 nM doxorubicin solution and naked AS and also both nanoparticles (Figs. 4 and 5). It is shown that the best transfection efficiency is obtained with chitosan/alginate nanoparticles with N/P ratio of 5. Mansouri et al. reported the same result for chitosan alone as DNA vector; they reported that the transfection efficiency increases when the amino group/phosphate group (N/P) ratio changes from 0.94:1 to 5.6:1, with a maximum rate of transfection at N/P ratio of 5.6:1 (Mansouri et al., 2004).

In another study Douglas et al. reported that chitosan/alginate nanoparticle-mediated transfection of 293T cells resulted in transfection levels as high as achieved with Lipofectamine™ after 48 h. The transfection efficiency of complexes prepared at a 5:1 N/P ratio was higher than with chitosan nanoparticles or naked plasmid. The improvement in transfection efficiency may be explained by the presence of alginate (Douglas et al., 2006).

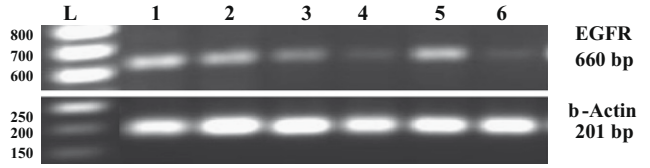

Fig. 4. EGFR mRNA expression in T47D cells. Effect of doxorubicin 250 nM (2), naked AS (3), nanoparticles alone (5) and AS loaded nanoparticles with N/P ratio of 5 (4) and 25 (6) and negative control RPMI (1) on the expression level of EGFR mRNA was analyzed in T47D cells after 4 h exposure using RT-PCR followed by agarose gel electrophoresis and EtBr staining. The L stands for ladder (DNA size Marker).

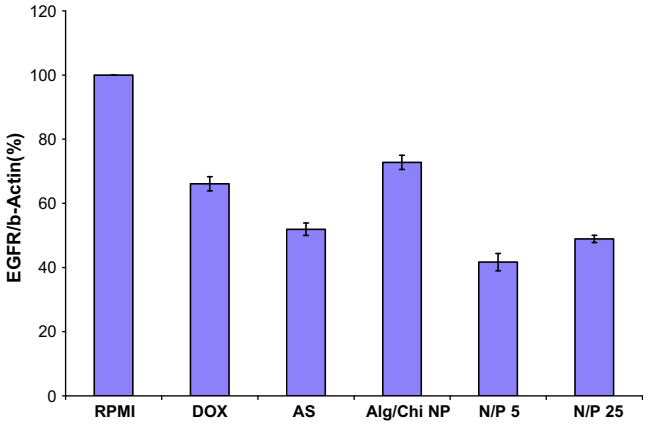

Fig. 5. Densitometric analysis of EGFR mRNA expression in T47D cells. Percent of mRNA expression of EGFR normalized to β-actin in the T47D cells exposed to doxorubicin 250 nM, naked AS, nanoparticles alone and AS loaded nanoparticles with N/P ratio of 5 and 25 determined by densitometry. All data are presented as mean of three different measurements ± SE.

3.4. Immunocytochemistry

Immunocytochemical analysis showed a decrease in EGFR protein expression which is a transmembrane protein in T47D cells following treatment with AS loaded chitosan/alginate nanoparticles. Decreasing of EGFR protein expression is more obvious in nanoparticles with N/P ratio of 5 than 25 or AS alone and even in cells which were treated with doxorubicin 250 nM (Fig. 6).

4. Conclusion

Optimized chitosan/alginate nanoparticles were prepared by pregel preparation method with 1:1 chitosan/alginate ratio and

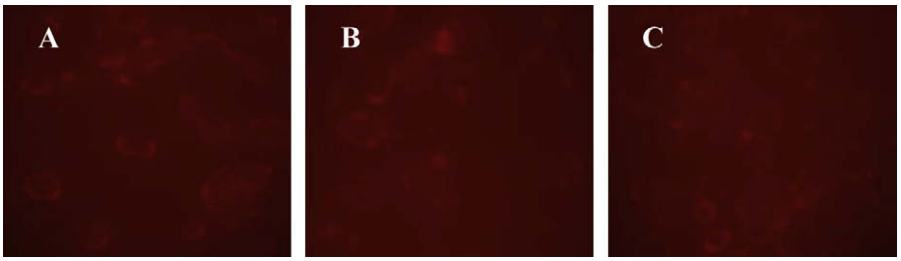

Fig. 3. Uptake of chitosan/alginate nanoparticles loaded with FITC-labeled EGFR antisense by T47D cells. Cellular uptake of the N/P ratios of 5 (B) and 25 (C) in comparison to the naked FITC-AS (A) in the T47D cells were evaluated after 4 h exposure under the fluorescent microscope. The magnification is 400×.

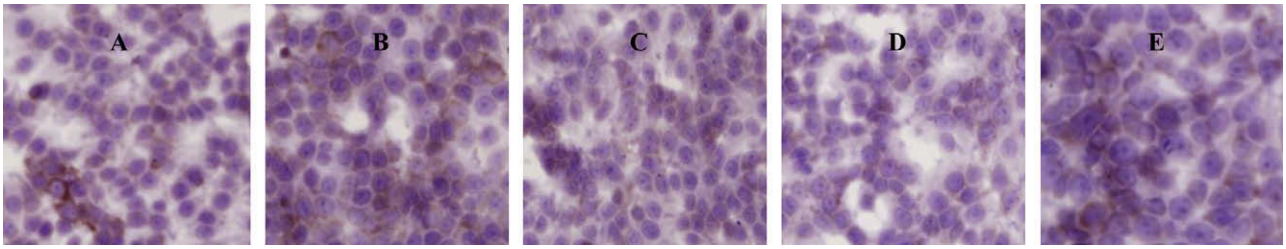

Fig. 6. Immunostaining of T47D cells with EGFR antibody. T47D cells treated for 4 h with doxorubicin 250 nM (B), naked AS (C), AS loaded nanoparticles with N/P ratios of 5 (D) and 25 (E) and negative control RPMI (A). Cells were immunostained with primary antibody for EGFR, visualized by LabVision detection system using AEC chromogen, mounted and studied under light microscope (magnification 100×).

N/P ratio of 5, providing desirable characteristics for antisense delivery. The T47D breast cancer cells treated with EGFR AS loaded chitosan/alginate nanoparticles showed down regulation of EGFR expression.

Transfection efficiency of nanoparticles with N/P ratio of 5 was significantly higher than N/P ratio of 25, naked AS and 250 nM doxorubicin. The improvement in transfection efficiency with polymeric carrier which is a non-toxic vehicle makes chitosan/alginate nanoparticles a suitable candidate for antisense delivery.

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