FABAD J. Pharm. Sci., 45, 1, 19-27, 2020

RESEARCH ARTICLE

Gene Delivery by Pei-Nanocomplex Into Breast and Colorectal Tumor Cell Lines, the Impacts of N/P Ratio, Size and Type of the Cell

Mohammad RASOOLIAN *, Seyed Younes HOSSEINI **, Hossein KHANAHMAD ***, Jamal SARVARI ****, Fatemeh RAHBARIZADEH *****, Ali Mohammad AMANI ******, Nasrollah ERFANI *******, Majid KHEIROLLAHI ******** °

Gene Delivery by Pei-Nanocomplex Into Breast and Colorectal Tumor Cell Lines, The Impacts Of N/P Ratio, Size and Type of The Cell

SUMMARY

The safety, low cost, ease of preparation and manipulation of non-viral vectors such as PEIs (polyethyleneimines) have made them a widespread tool for gene delivery despite their poor efficiency compared to viral vectors. We used PEI in comparison with liposome reagent in combination with pEGFP-C1 to evaluate transfection efficiency and cytotoxicity in various cancer cell lines including MCF-7, SW-480, and CT-26. The N/P ratio (nitrogen groups of polymer/ phosphate groups of nucleic acid) 10:1 for PEI 2000 and PEI 25000 was selected as the most appropriate N/P ratio for transfection based on cell viability and transfection efficiency. The cell types received the nanoparticles and lipofectamines with different efficiencies according to the following relationship: MCF-7 (P<0.001)>SW-480 (P<0.001)>CT-26 (P<0.001). PEI 25000 acted as a non-viral vector better than PEI 2000 and lipofectamineTM 2000 (P<0.001) to transfect of pEGFP-C1 into MCF-7 breast cancer cell line, whereas PEI 2000 and lipofectamineTM 2000 were the better choice for plasmid transfection compared to PEI 25000 in SW-480 and CT-26 colorectal cancer cell lines. The results showed that by decreasing the size and increasing the charge of PEI 2000 and PEI 25000 nanoparticles the gene delivery was increased for colorectal cancer cell lines including SW-480 and CT-26. However, PEI 2000 and lipofectamine were more effective at entering colon cancer cells than PEI 25000. Increasing the size and charge associated with the nanoparticles, improved the expression level of GFP in MCF-7 breast cancer cells. Results of this study showed that evaluation of non-viral vectors on cancer cell types avoids wasting time and duplication studies.

Key Words: *Gene delivery, Nanoparticle, Liposome, MCF-7, SW-480, CT-26.*

Pei-Nanocomplex tarafından Meme ve Kolorektal Tümör Hücre Hatlarında Gen Dağıtımı , N / P Oranının, Hücrenin Boyutu ve Türünün Etkileri

ÖZ

Viral vektörlere kıyasla zayıf verimlerine rağmen güvenlik, düşük maliyet, hazırlık kolaylığı ve manipülasyonları PEI'ler (polietileniminler) gibi viral olmayan vektörleri gen dağıtımı için yaygın bir araç haline getirmiştir. MCF-7, SW-480 ve CT-26 dahil olmak üzere çeşitli kanser hücre hatlarında transfeksiyon etkinliği ve sitotoksisiteyi değerlendirmek için pEGFP-C1 ile kombinasyon halinde lipozom reaktifi ile karşılaştırmak amacıyla PEI'yi kullandık. Hücre canlılığı ve transfeksiyon verimine dayalı transfeksiyon amacıyla PEI 2000 ve PEI 25000 için N/P oranı (azot grupları / polimer nükleik asit grubu) en uygun 10: 1 olarak seçildi. Hücre tipleri, nanopartikülleri ve lipofektaminleri farklı etkinliklerde MCF-7 (P<0.001)> SW-480 (P<0.001)> CT-26 (P<0.001) ilişkisine göre aldı. PEI 25000, pEGFP-C1'in MCF-7 göğüs kanseri hücre hattına transfekte edilmesi için PEI 2000 ve lipofektaminTM 2000'den daha iyi viral olmayan bir vektör olarak etki ederken (P <0.001), PEI 2000 ve lipofektaminTM 2000, plazmid transfeksiyonu için SW-480 ve CT-26 kolorektal kanser hücre hatlarında PEI 25000'e daha iyi bir seçimdir. Sonuçlar, PEI 2000 ve PEI 25000 nanoparçacıklarının büyüklüğünü azaltarak ve yükünü artırarak gen dağıtımının SW-480 ve CT-26 dahil olmak üzere kolorektal kanser hücre hatları için arttığını göstermiştir. Bununla birlikte, PEI 2000 ve lipofektamin, kolon kanseri hücrelerine girmede PEI 25000'den daha etkili bulunmuştur. Nanopartiküller ile ilişkili boyut ve yükün artırılması, MCF-7 göğüs kanseri hücrelerinde GFP'nin ifade düzeyini yükseltmiştir. Bu çalışmanın sonuçları viral olmayan vektörlerin kanser hücresi tipleri üzerinde değerlendirilmesinin, zaman kaybını ve fazladan çalışmaları önlediğini göstermiştir.

Anahtar Kelimeler: *Gen dağıtımı, Nanoparçacık, Lipozom, MCF-7, SW-480, CT-26.*

Received: 04.04.2019
Revised: 03.10.2019
Accepted: 06.10.2019

* ORCID: 0000-0003-3009-6245, Department of Genetics and Molecular Biology, School of Medicine, Isfahan University of Medical Sciences, Isfahan, Iran.
** ORCID: 0000-0002-4336-8169, Bacteriology and Virology Department, School of Medicine, Shiraz University of Medical Sciences, Shiraz, Iran.
*** ORCID: 0000-0002-5573-3557, Department of Genetics and Molecular Biology, School of Medicine, Isfahan University of Medical Sciences, Isfahan, Iran.
**** ORCID: 0000-0002-2259-0836, Bacteriology and Virology Department, School of Medicine, Shiraz University of Medical Sciences, Shiraz, Iran.
***** ORCID: 0000-0003-0890-8366, Department of Medical Biotechnology, School of Medical Sciences, Tarbiat Modares University, Tehran, Iran.
****** ORCID: 0000-0002-7034-3071, Department of Medical Nanotechnology, School of Advanced Medical Sciences and Technologies, Shiraz University of Medical Sciences, Shiraz, Iran.
******* ORCID: 0000-0002-4158-9128, Shiraz Institute for Cancer Research, School of Medicine, Shiraz University of Medical Sciences, Shiraz, Iran.
******** ORCID: 0000-0003-1981-1340, Department of Genetics and Molecular Biology, School of Medicine, Isfahan University of Medical Sciences, Isfahan, Iran.

° Corresponding Author; Majid Kheirollahi
Phone numbers: +989133286113, E-mail: mkheirollahi@med.mui.ac.ir

INTRODUCTION

The non-viral vectors considered as possible alternatives to viral gene delivery (Cheraghi, Nazari, Alipour, Majidi, & Hosseinkhani, 2016). The viral vectors can transfect cells with high efficiency, but there are concerns associated with viral vector systems such as immunogenicity, cytotoxicity and limitation due to their time-consuming and costly preparation, inability to transfer siRNA and other novel systems like CRISPR/Cas into cells encouraged the studies toward the use of non-viral vectors to transfer exogenous DNA to target cells (Bharali et al., 2005; Check, 2002; Cheraghi et al., 2016; Verma & Somia, 1997). Due to the ease of preparation and manipulation and to overcome problems of cost, immunogenicity, safety, and mutagenesis, various non-viral carriers have been developed (Felgner, 1997; Mintzer, 2008). These vectors include polyethyleneimine (PEI), poly-L-Lysine, calcium phosphate, dendrimer, protamine, and lipofectamine (Cheraghi, 2017; Kircheis, Wightman, & Wagner, 2001; Wagner, Ogris, & Zauner, 1998).

Polyethyleneimine (PEI) is used as a cationic polymer as a highly efficient non-viral vector, which has been confirmed to be an effective transfection agent both *in vitro* and *in vivo* (Coll et al., 1999; Godbey, Wu, & Mikos, 1999; Huh et al., 2007). One of the most important physicochemical properties of polymer-based gene delivery vehicles is the N/P ratio. The N/P ratio is the ratio of positively-charged polymer amine (N=nitrogen) groups to negatively-charged nucleic acid phosphate (P) groups in polymer/nucleic acid complex that can influence many other properties such as its stability, surface charge, and size (Bragonzi et al., 2000). The high positive charge density of PEI due to ammonium ions induces electrostatic affinity to the negative charge of DNA (phosphate group) and condenses compact particles (Huh et al., 2007). The proton sponge effect (osmotic imbalance) of PEI by creating instability in endosomes causes high transfection activity after being taken up by cells (Boussif et al., 1995; Huh et al., 2007; Kichler, Leborgne, Coeytaux, & Danos, 2001). The PEI 2000 and PEI 25000 used in our research had branches. The presence of primary, secondary and tertiary amines in the ratio 1:2:1 in branched PEI provide buffering capability, which leads to endosomo lysis and release of PEI/DNA complexes in the cytosol (Akinc, Thomas, Klibanov, & Langer, 2005).

Cellular uptake and intra-cellular transport including endosomal escape, and cytoplasmic transport of particles depends on the cell type, as well as cargo type (Figueroa et al., 2017). Increase in concentration and decrease in size of pDNA/material may influence the endocytosis pathway and kinetic of internalization (Luo et al., 2015). The micropinocytosis, endocytosis, phagocytosis, direct diffusion or adhesive interactions are the mechanisms of transport of nano-particles and solutes across cell membranes (de Lima, Seabra, & Durán, 2012). The ways in which nanoparticles are internalized by cells are determined by their physical and chemical properties, such as size, surface charge, shape, and composition that size was found to be an essential factor (Gratton et al., 2008; Wu, Guo, Liu, Liu, & Xie, 2019). For efficient transfection and endocytosis, the surface charge and size of nanocomplexes must be positive and smaller than 500 nm, respectively (Decuzzi & Ferrari, 2008).

Lipofectamine reagent as a gold-standard for the safe delivery of exogenous DNA or RNA into cells is a cation-lipid formulation transfection reagent that forms liposomes in aqueous media (Cardarelli et al., 2016). Both PEI and lipofectamine™ 2000 mediated endocytosis involved clathrin-dependent, caveolae-mediated and macropinocytosis pathways in transfection (Luo et al., 2015).

In this study, branched PEI 2000 and PEI 25000 were compared with lipofectamine 2000™ reagent (as control) to investigate the transfection efficiency in breast and colorectal tumor cells and to determine the best N/P ratio for this purpose.

MATERIALS AND METHODS

The branched PEI (2000 and 25000) and Lipofectamine 2000™ reagent were purchased from Sigma-Aldrich (USA) and Invitrogen (USA). MCF-7 (human breast adenocarcinoma cell line), SW480 (human colon cancer cell line), CT-26 (murine colorectal carcinoma cell line from a BALB/c mouse) were purchased from Pasteur Institute of Iran.

Preparation of Plasmid DNA

Plasmid DNA (pDNA) containing enhanced green fluorescent protein gene (pEGFP-C1) was amplified in *Escherichia coli* DH5 strain, isolated and purified using a mini-prep kit (MN, Germany) according to the manufacturer's protocol. The concentration and integrity of DNA were determined by measuring *NanoDrop*™ spectrophotometer (ND1000, USA) at 260 nm as well as electrophoresis.

Cell Culture

CT-26 and SW480 were cultured in RPMI (Roswell Park Memorial Institute) medium and MCF-7 in DMEM (Dulbecco's Modified Eagle's medium)

supplemented with 10% fetal bovine serum, 2 mM L-glutamine, 100 units/ml penicillin, and 100 µg/ml streptomycin. Cells were grown to confluency at 37 °C in a 5% CO_2 humidified atmosphere.

Nano-Complexes Evaluation

N/P (nitrogen/phosphorus) ratio factor was used to study of nanoparticle properties of PEI 2000 and PEI 25000 with pEGFP-C1 plasmid. To investigate the formation of nanoparticle, at first, gel retardation of nanocomplexes by PEI was done. pDNA containing pEGFP-C1 was mixed with varying amounts of branched PEI 2000 and 25000 at a concentration of 30 mg/ml by diluting in distilled water and checked by transilluminator and zeta sizer. For transfection, the pDNA-PEI reagent mixtures were incubated in appropriate medium without FBS at room temperature for 30 minutes. Mixed DNA/polymer nanoparticles were examined using 1% agarose gel electrophoresis to check the complex formation in transilluminator. After the preparation of nanocomplex, the potential and size of PEI 2000 and 25000 complexes were measured using NANO-flex® (Microtrac, USA) zeta potential analyzer and Zeta sizer (Microtrac, Nano-flex® 180°, USA).

***In Vitro* Transfection**

Cells were seeded at a density of 1×10^5 cells/well in 24-well plates on the day before transfection and grown in the appropriate medium with 10% fetal bovine serum. The cell lines were 80% to 90% confluent at the time of transfection. Each prepared nanoparticle solution and complex pDNA containing pEGFP-C1 with lipofectamine 2000™ were incubated for 30 minutes at room temperature and added to the cells. Transfection was performed in complete medium for 4 hours. The medium was replaced with fresh complete medium. Control group was transfected using Lipofectamine™ 2000 (Invitrogen,USA). Gene expression was assayed 24 hours post-transfection through observing the EGFP expression by fluorescence microscopy.

MTT Assays

The MTT cell assay (Kiazist Inc. Iran) was performed to evaluate cell viability and proliferation. Cell lines including MCF-7, SW480 and CT-26 were seeded on 96 wells culture plate with a cell density of $5\text{-}10\times10^3$ and incubated for 24 hrs. The amount of pEGFP-C1 plasmid was 200 ng and PEI 2000 and 25000 was set at N/P ratios 5:1, 10:1 and 20:1 to determine the best efficiency of transfecting and the lowest cytotoxicity for various cell lines. The nanocomplexes were made in FBS-free medium and added to each well after 30 minutes incubation. After 4 hours, the culture medium was replaced with 150 µl fresh culture medium. Then to perform the MTT test, after 24 h, 10 µl of 5 mg/ml of MTT reagent in sterile phosphate-buffered saline buffer was added to each well. Following incubation for 4 hours at 37°C, the medium was removed and formazan solubilizer 100 µl of dimethyl sulfoxide was added, and the samples were incubated at 37 °C for 20 minutes under shaking. By microplate reader, the optical absorption was measured at 570 nm, which was shown as mean ± SD in triplicate.

Statistical Analysis

All tests were carried out in triplicate. The SPSS (v.16.0) and Microsoft EXCEL software were used for statistical analysis and graphing. The data were reported as mean ± SD and statistical significance was analyzed by one-way ANOVA (Tukey's post-test) and two-way ANOVA (Scheffe's post-test). *P* values 0.05 was considered statistically significant.

RESULTS

Nano-Complexes Formation, Size and Zeta Potential Analysis

At first, the nano-complexes formation was assessed through gel retardation assay and reduced electrophoretic mobility of the nano-complex in the gel matrix. In addition, the particle sizes of PEI 2000-pEGFP-C1 and PEI 25000-pEGFP-C1 in the 5:1, 10:1 and 20:1 N/P ratios are shown in figure 1. The size of PEI 2000-pEGFP-C1 and PEI 25000-pEGFP-C1 nanoparticles were determined to be 194-344 nm and 370-478 nm, respectively, indicating that the nanostructure is well formed. It also showed that by increasing N/P ratio (PEI content), the size of PEI 2000-pEGFP-C1 and PEI 25000-pEGFP-C1 nanoparticles significantly increased, so that at 20:1 N/P ratio the largest nanoparticles were formed. Moreover, by increasing the molecular weight of PEI, the structure of nanoparticles was enlarged as shown in figure 1.

The positive zeta potential of the nanoparticles containing branched PEI 2000-pEGFP-C1 and PEI 25000-pEGFP-C1 were determined at various N/P ratios, as depicted in figure 2. The net charge of nanocomplexes determined to be positive regardless of PEI weight. The results showed that by increasing the N/P ratio, the positive potential of PEI 2000-pEGFP-C1 and PEI 25000-pEGFP-C1 nanoparticles increased constantly (Figure 2).

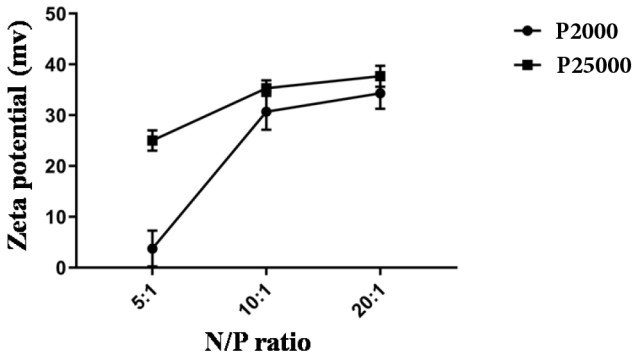

Figure 1. The particle size analysis of PEI-pEGFP-C1. The size of PEI 2000-pDNA (filled circles) and PEI 25000-pDNA (filled squares) at various N/P ratios were determined by zeta sizer. Results represent the mean±SD of three measurements.

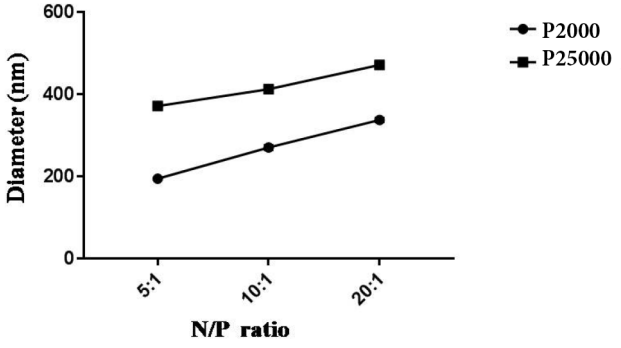

Figure 2. The zeta potential analysis of PEI-pEGFP-C1. PEI 2000-pDNA (filled circles) and PEI 25000-pDNA (filled squares) at various N/P ratios are shown. Results are the mean±SD of three measurements.

Cytotoxicity Measured by MTT Test

To determine the best N/P ratio of nanoparticles with minimal cytotoxicity, MTT assay was performed at 5:1, 10:1 and 20:1 N/P ratios for Human embryonic kidney (HEK) 293 cells. As shown in Figure 3, an increase in the molecular weight of PEI, in turn, induced a more cytotoxic effect on 293T cells. By increasing the N/P ratios of PEI 2000 and PEI 25000, the viability of cells dropped, with a 20:1 ratio being the most affected. PEI 2000 had low cytotoxicity in combination with pEGFP-C1 plasmid at 5:1 and 10:1 N/P ratios so that cell survival did not differ significantly with control (95%), 91% and 88%, respectively. While at 20:1 N/P ratio, the cytotoxicity rate (54%) increased (P<0.001) compared to control and 5: 1 and 10: 1 N / P ratios. The cytotoxicity of PEI 25000 at 5:1 and 10:1 N/P ratios was similar to that of PEI 2000, with no significant difference with control cells. Cell viability was 89% and 86%, respectively (Figure 3). A significant increase (P<0.001) in cytotoxicity of P 25000 was observed at 20:1 N/P ratio (44%) compared to the control group as well as other 5:1 and 10:1 N/P ratios (Figure 3). Lipofectamine also did not show a significant difference with cell viability of 92% compared to the control group.

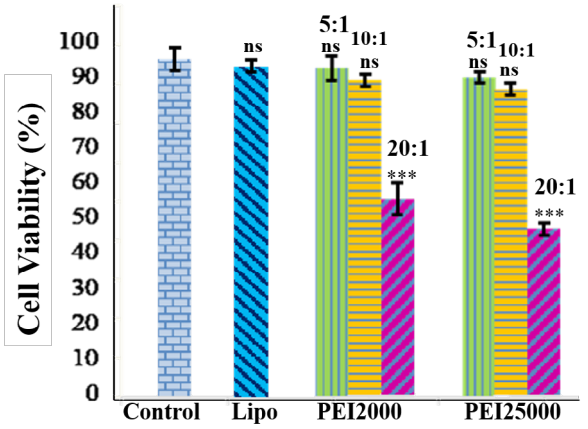

Figure 3. The cell viability test. MTT assay determined cytotoxicity of nanoparticles including PEI 2000 and PEI 25000 at 5:1, 10:1, and 20:1 N/P ratios for 293 HEK transfection. The differences among groups were assessed by one-way ANOVA (followed by Tukey's test). The mean viability of cells was compared with control cell (mean±SD, n=3). Lipo: lipofectamine, ns: non-significant, ***: $P<0.001$.

Transfection Efficiency

After transfection, GFP signals were analyzed by fluorescent microscopy to obtain relative transfection rates in cell lines including MCF-7, SW4-80 and CT-26(Figure 4(A)). In order to determine the best PEI ratios of 2000 and 25000, the nanoparticles were examined at 5:1, 10:1 and 20:1 N/P ratios. The transfection efficiency enhanced dramatically with increasing N/P ratios, with the highest 20:1 ratio but also the highest cytotoxic effect in MTT assay. Therefore, the 10: 1 ratio of PEI 2000 and 25000 was selected as the most reliable ratio of nanoparticles for transfection experiments. Here, lipofectamine™ 2000 was also used as the positive control. The transfection capabilities of PEI nanoparticles (PEI 2000-pEGFP-C1 and PEI 25000-pEGFP-C1) and lipofectamineTM 2000 in MCF-7, CT-26 and SW-480 showed a completely different pattern of function with the following relationship. The transfection capabilities of the PEI nanoparticles (PEI 2000-pEGFP-C1 and PEI 25000-pEGFP-C1) and lipofectamine™ 2000 in MCF-7, CT-26 and SW-480 showed a completely different pattern of efficiency with the following relationship: MCF-7 ($P<0.001$)>SW-480 ($P<0.001$)>CT-26 ($P<0.001$) (Figure 4A, 4B). In general, MCF-7 and SW-480 exhibited more suitable cell line for receiving plasmids via nanocomplexes and lipofectamine™ 2000. On the other hand, CT-26 showed the lowest transfection rate following nanocomplex and lipofectamine™ 2000 treatment. In MCF-7 cell line, there were significant differences in transfection efficiency between lipofectamine and PEI 25000 (lowest transfection) and between PEI 2000 and PEI 25000 ($P<0.001$), but there was no significant difference ($P>0.05$) between lipofectamine and PEI 2000 (Figure 4(B)). Transfection efficiency of Lipofectamine™ 2000 in CT-26 cell line was as follows: lipofectamine™ 2000, PEI 2000 ($P<0.001$) > PEI 25000 (Figure 4(B)). The highest expression of green fluorescent protein (GFP) in SW-480 induced by lipofectamine™ 2000 and was significantly different with PEI 25000 ($P<0.001$), whereas it was no significantly different with PEI 2000 (Figure 4(B)). The number of SW-480 cells expressing GFP by PEI 2000 was more than PEI 25000 ($P<0.001$) (Figure 4(B)).

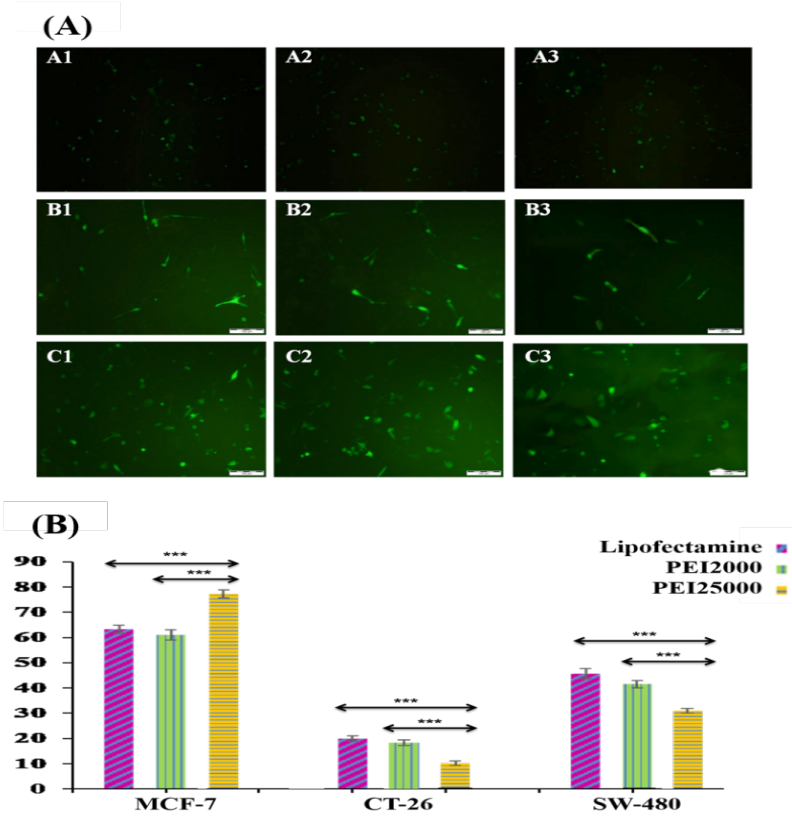

Figure 4. The transfection results by different nano-complexes. **(A)** The fluorescence microscopy results of transfection of pEGFP-C1 using PEI 2000, PEI 25000 and Lipofectamine™ 2000 on MCF-7, CT-26 and SW480. A1: MCF-7 transfection by lipofectamine, A2: MCF-7 transfection by PEI 2000, A3: MCF-7, transfection by PEI 25000, B1: CT-26, transfection by lipofectamine, B2: CT-26, transfection by PEI 2000, B3: CT-26, transfection by PEI 25000, C1: SW-480, transfection by lipofectamine, C2: SW-480, transfection by PEI 2000, C3: SW-480, transfection by PEI 25000. **(B)**. Differences in the rate of Transfection efficiency by nanoparticles and Lipofectamine™ 2000 for three cell lines. Gene expression was assayed 24 hours post-transfection through observation of EGFP expression by fluorescence microscopy. The differences between groups were evaluated by two-way ANOVA (followed by scheffe's test). All assays were done in triplicates and expressed as mean ± SD. *** indicates significantly different ($P \leq 0.001$).

DISCUSSION

Ease of preparation and manipulation of PEIs (polyethyleneimines) have made them a widespread tool for gene delivery (Felgner, 1997; Mintzer MA, 2008). PEI is used as a highly efficient delivery vector of plasmid, which exhibited efficiency both *in vitro* and *in vivo* (Coll et al., 1999; Godbey et al., 1999). However, because of the different efficiency of PEIs and its cytotoxic effects, evaluation to find the appropriate structure and proper safety in different cell lines should be evaluated with a control transfection agent such as lipofectamine 2000. Here, the size and potential charge of PEI 2000 and 25000 nanocomplexes were investigated and then the cytotoxicity, as well as efficiency, was evaluated on colorectal and breast carcinoma cell lines.

In the first step, the binding capacity of cationic ammonium of PEI to the negative charge of pDNA and nanoparticle formation via gel retardation assay was examined. Moreover, weak safe stain signals at various N/P ratios represent tight binding between pDNA and PEI, leading to the release safe stain.

In order to transport nano-particles and liposomes across cell membranes, endocytosis is the major route, which is classified as pinocytosis and phagocytosis. Pinocytosis pathway of DNA/material particles that present in all types of cells is classified into four forms including clathrin-dependent endocytosis, caveolae-dependent endocytosis, macropinocytosis, and clathrin-independent and caveolae-independent endocytosis (Rappoport, 2008; Wang, Byrne, Napier, & DeSimone, 2011). Both PEI and lipofectamine™

2000 transfect plasmids or DNA from endocytosis pathways including clathrin-dependent, caveolae-mediated and macropinocytosis pathways (Luo et al., 2015).

For efficient transfection and endocytosis, the surface charge and size of nanocomplexes must be positive and smaller than 500 nm, respectively (Decuzzi & Ferrari, 2008). In this study, potential charge of PEI 2000 and 25000 in all ratios was positive (Figure 2). In addition, the size of branched PEI 2000-pEGFP-C1 and PEI 25000-pEGFP-C1 at various N/P ratios were measured 194-344 nm and 370-478 nm, respectively (Figure 1).

In agreement with previous studies, by increasing N/P ratio (PEI content), the size of PEI 2000-pEGFP-C1 and PEI 25000-pEGFP-C1 nanoparticles significantly increased and also by increasing the molecular weight of PEI, the size of nanoparticles would be larger (Figure 1)(Cheraghi R, 2017; Morimoto et al., 2003). Coordinated with other studies, by increasing the N/P ratio, the positive potential of PEI 2000-pEGFP-C1 and PEI 25000-pEGFP-C1 nanoparticles increased constantly (Figure 2) (Cheraghi R, 2017; Kawakami, Ito, Charoensit, Yamashita, & Hashida, 2006).

Reduced of cytotoxicity of PEI is an important factor for successful gene delivery. The strong positive charge of PEI leads to electrostatic interaction with the negatively charged cell surface membrane that induces cellular harm (Prevette, Mullen, & Holl, 2010). PEIs cytotoxicity analysis with respect to various N/P ratios consisting of 5:1, 10:1 and 20:1 revealed that a 10:1 ratio was the most appropriate N/P ratio.

It is difficult to compare the efficiency of transfection in different studies. Many experiments reported in the literature are difficult to compare because different cell types and different transfection agents have been used.

In this study, the transfection efficiency of the nanoparticles (PEI 2000-pEGFP-C1 and PEI 25000-pEGFP-C1) and pEGFP-C1 in combination with lipofectamine™ 2000 were investigated in MCF-7, CT-26 and SW-480 cell lines (Figure 4(A), 4(B)). In General, MCF-7 exhibited a most permeable cell to receive plasmids by nanocomplexes and lipofectamine™ 2000 (>60% transfection rate). PEI 25000 acted better as a non-viral vector rather than PEI 2000 and lipofectamine™ 2000 (P<0.001) to transfect pEGFP-C1 into MCF-7 cell line (Figure 4(A), 4(B)).

SW-480 cells showed less transfection than MCF-7 compared to MCF-7 treated with nanocomplex and lipofectamin. SW-480 cells showed lower transfection rates compared to MCF-7 treated with nanocomplex and lipofectamine™ 2000 (all vectors>32% transfection rate) as consistent with other studies (Gray Z, 2018). The efficiency of plasmid transfection by lipofectamine™ 2000 in SW480 cell line was higher than PEI 25000 (P<0.001), and also PEI 2000 was higher than PEI 25000 (P<0.001), so it seems that selection of PEI 2000 and lipofectamine™ 2000 as synthetic non-viral vectors are the better choices for plasmid transfection in SW-480 cells. In terms of cost, PEI 2000 can be a more appropriate option (Figure 4(A), 4(B)).

CT-26 cells as "difficult to transfect" cell line demonstrated the lowest transfection rate following nanocomplex and lipofectamine™ 2000 treatment (all vector<21% transfection) (Benns, Mahato, & Kim, 2002; Figueroa et al., 2017). Several groups have reported the lower transfectability of CT-26 cells (Benns et al., 2002; Fong et al., 2004; Han et al., 2015). The number of CT-26 cells with expressed GFP by lipofectamine™ 2000 were higher than PEI 25000 (P<0.001), whereas there was no significant differences between lipofectamine™ 2000 and PEI 2000, so it seems lipofectamine™ 2000 and PEI 2000 are appropriate synthetic non-viral vectors for gene delivery in CT-26 cells. Among the two, PEI 2000 is also a better option because of the cheaper options (Figure 4(A), 4(B)).

CONCLUSION

In this study, MCF-7 cells had the highest plasmid transfection among all cell lines. SW-480 cells also received significantly more plasmids than CT-26 cells, which may be due to their greater efficiency in uptake, trafficking and / or transcribing the DNA. It can be concluded that CT-26 cells, which are "difficult to transfect" cells, pose many barriers to the transfect of plasmids into the cell. Finally, PEIs did not damage the cells, the gene delivery rate for the colon cancer cell lines including SW-480 and CT-26 increased by decreasing the size and increasing the charge of PEI 2000 and PEI 25000 nanoparticles. However, the PEI 2000 and lipofectamine are more effective than the PEI 25000 in entering cancer intestinal cells. While increasing the size and charge of nanoparticles, the level of GFP expression in the breast cancer cell MCF-7 increased.

ACKNOWLEDGMENTS

The article has been derived from a part of the Ph.D. thesis grant number: 395535, from Isfahan University of Medical Sciences, Isfahan, Iran.

CONFLICT OF INTEREST

The authors declare no conflict of interest, financial or otherwise.

REFERENCES

Akinc, A., Thomas, M., Klibanov, A. M., & Langer, R. (2005). Exploring polyethylenimine-mediated DNA transfection and the proton sponge hypothesis. *Journal of Gene Medicine*, *7(5*), 657-663.

Benns, J. M., Mahato, R. I., & Kim, S. W. (2002). Optimization of factors influencing the transfection efficiency of folate-PEG-folate-graft-polyethylenimine. *Journal of Control Release, 79*(1-3), 255-269.

Bharali, D. J., Klejbor, I., Stachowiak, E. K., Dutta, P., Roy, I., Kaur, N., . . . Stachowiak, M. K. (2005). Organically modified silica nanoparticles: a nonviral vector for in vivo gene delivery and expression in the brain. *Proceedings of the National Academy of Sciences of the United States of America, 102*(32), 11539-11544.

Boussif, O., Lezoualc'h, F., Zanta, M. A., Mergny, M. D., Scherman, D., Demeneix, B., & Behr, J. P. (1995). A versatile vector for gene and oligonucleotide transfer into cells in culture and in vivo: polyethylenimine. *Proceedings of the National Academy of Sciences of the United States of America, 92*(16), 7297-7301.

Bragonzi, A., Dina, G., Villa, A., Calori, G., Biffi, A., Bordignon, C., . . . Conese, M. (2000). Biodistribution and transgene expression with nonviral cationic vector/DNA complexes in the lungs. *Gene Therapy, 7*(20), 1753.

Cardarelli, F., Digiacomo, L., Marchini, C., Amici, A., Salomone, F., Fiume, G., . . . Caracciolo, G. (2016). The intracellular trafficking mechanism of Lipofectamine-based transfection reagents and its implication for gene delivery. *Scientific Reports*, *6*, 25879.

Check, E. (2002). A tragic setback. *Nature, 420*(6912), 116-118.

Cheraghi R, A. M., Nazari M, Hosseinkhani S. (2017). Optimization of conditions for gene delivery system based on PEI. *Nanomedicine Journal, 4*(1), 8-16.

Cheraghi, R., Nazari, M., Alipour, M., Majidi, A., & Hosseinkhani, S. (2016). Development of a Targeted anti-HER2 scFv Chimeric Peptide for Gene Delivery into HER2-Positive Breast Cancer Cells. *International Journal of Pharmaceutics, 515*(1-2), 632-643.

Coll, J. L., Chollet, P., Brambilla, E., Desplanques, D., Behr, J. P., & Favrot, M. (1999). In vivo delivery to tumors of DNA complexed with linear polyethylenimine. *Human Gene Therapy, 10*(10), 1659-1666.

de Lima, R., Seabra, A. B., & Durán, N. (2012). Silver nanoparticles: a brief review of cytotoxicity and genotoxicity of chemically and biogenically synthesized nanoparticles. *Journal of Applied Toxicology, 32*(11), 867-879.

Decuzzi, P., & Ferrari, M. (2008). The receptor-mediated endocytosis of nonspherical particles. *Biophysical Journal*, *94*(10), 3790-3797.

Felgner, P. L. (1997). Nonviral strategies for gene therapy. *Scientific American, 276*(6), 102-106.

Figueroa, E., Bugga, P., Asthana, V., Chen, A. L., Stephen Yan, J., Evans, E. R., & Drezek, R. A. (2017). A mechanistic investigation exploring the differential transfection efficiencies between the easy-to-transfect SK-BR3 and difficult-to-transfect CT26 cell lines. *Journal of Nanobiotechnology, 15*(1), 36.

Fong, S., Liu, Y., Heath, T., Fong, P., Liggitt, D., & Debs, R. J. (2004). Membrane-permeant, DNA-binding agents alter intracellular trafficking and increase the transfection efficiency of complexed plasmid DNA. *Molecular Therapy*, *10*(4), 706-718.

Godbey, W. T., Wu, K. K., & Mikos, A. G. (1999). Poly(ethylenimine) and its role in gene delivery. *Journal of Control Release, 60*(2-3), 149-160.

Gratton, S. E., Ropp, P. A., Pohlhaus, P. D., Luft, J. C., Madden, V. J., Napier, M. E., & DeSimone, J. M. (2008). The effect of particle design on cellular internalization pathways. *Proceedings of the National Academy of Sciences, 105*(33), 11613-11618.

Gray Z, D. Y., Tabarraei A, Moradi A. (2018). Optimization of SW480 Colon Cancer Cells Transfection with Lipofectamine 2000. *Medical Laboratory Journal, 12*(4), 12-16.

Han, X., Liu, Z., Jo, M. C., Zhang, K., Li, Y., Zeng, Z., . . . Qin, L. (2015). CRISPR-Cas9 delivery to hard-to-transfect cells via membrane deformation. *Science Advances, 1*(7), e1500454.

Huh, S. H., Do, H. J., Lim, H. Y., Kim, D. K., Choi, S. J., Song, H., . . . Kim, J. H. (2007). Optimization of 25 kDa linear polyethylenimine for efficient gene delivery. *Biologicals, 35*(3), 165-171.

Kawakami, S., Ito, Y., Charoensit, P., Yamashita, F., & Hashida, M. (2006). Evaluation of proinflammatory cytokine production induced by linear and branched polyethylenimine/plasmid DNA complexes in mice. *Journal of Pharmacology and Experimental Therapeutics, 317*(3), 1382-1390.

Kichler, A., Leborgne, C., Coeytaux, E., & Danos, O. (2001). Polyethylenimine-mediated gene delivery: a mechanistic study. *The Journal of Gene Medicine*, *3*(2), 135-144.

Kircheis, R., Wightman, L., & Wagner, E. (2001). Design and gene delivery activity of modified polyethylenimines. *Advanced Drug Delivery Reviews, 53*(3), 341-358.

Luo, J., Li, C., Chen, J., Wang, G., Gao, R., & Gu, Z. (2015). An efficient method for in vitro gene delivery via regulation of cellular endocytosis pathway. *International Journal of Nanomedicine*, *10*, 1667-1678.

Mintzer, M. A., Simanek, E. E. (2008). Non-viral Vectors for Gene Delivery. *Chemical reviews, 109*, 259–302.

Morimoto, K., Nishikawa, M., Kawakami, S., Nakano, T., Hattori, Y., Fumoto, S., . . . Hashida, M. (2003). Molecular weight-dependent gene transfection activity of unmodified and galactosylated polyethyleneimine on hepatoma cells and mouse liver. *Molecular Therapy*, *7*(2), 254-261.

Prevette, L. E., Mullen, D. G., & Holl, M. M. (2010). Polycation-induced cell membrane permeability does not enhance cellular uptake or expression efficiency of delivered DNA. *Molecular Pharmaceutics, 7*(3), 870-883.

Rappoport, J. Z. (2008). Focusing on clathrin-mediated endocytosis. *Biochemical Journal, 412*(3), 415-423.

Verma, I. M., & Somia, N. (1997). Gene therapy -- promises, problems and prospects. *Nature, 389*(6648), 239-242.

Wagner, E., Ogris, M., & Zauner, W. (1998). Polylysine-based transfection systems utilizing receptor-mediated delivery. *Advanced Drug Delivery Reviews*, *30*(1-3), 97-113.

Wang, J., Byrne, J. D., Napier, M. E., & DeSimone, J. M. (2011). More effective nanomedicines through particle design. *Small, 7*(14), 1919-1931.

Wu, M., Guo, H., Liu, L., Liu, Y., & Xie, L. (2019). Size-dependent cellular uptake and localization profiles of silver nanoparticles. *International Journal of Nanomedicine, 14*, 4247.