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FeCo-Chitosan / DNA nanoparticles for gene transfer to MCF-7 breast cancer cells: preparation and characterization

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Running head: Nanoparticles for gene delivery to MCF7- breast cancer cells

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ABSTRACT

Background: Researchers have seen gene therapy as one of the most important techniques for treating illnesses including cancer and a range of genetic problems in recent years. The capacity of FeCo-Chitosan nanoparticles for gene transport into MCF-7 cells was explored in this study

Methods: FeCo-Chitosan/DNA nanoparticles were prepared. Then, physicochemical features of nanoparticles, were assessed using SEM. Also, biological features of the nanoparticles including biocompatibility, DNA protection, DNA release, and gene transfer capacity to MCF-7 cells were studied,.

Results: Results showed that FeCo-Chitosan / DNA nanoparticles exhibited a spherical shape with an average size of around 200 nm. The zeta potential of the FeCo-Chitosan/DNA complex increased with increasing the concentration of FeCo-Chitosan nanoparticles in the FeCo-Chitosan/DNA complex. Electrophoretic analyses showed that FeCo-Chitosan/DNA nanoparticles protects DNA against nuclease degradation and ultrasonic damage. Also, MTT test revealed that FeCo-Chitosan nanoparticles had a good biocompatibility.

Conclusions: FeCo-Chitosan nanoparticles may safely transfer and release DNA to MCF-7 cells, according to fluorescence microscopy and flow cytometry studies. These findings also revealed that increasing the concentration of FeCo-Chitosan in the FeCo-Chitosan/DNA complex improved gene transfer efficiency.

Keywords: Chitosan, FeCo, Gene delivery, Magnetic properties, Cancer, Nanopar.

INTRODUCTION:

In recent years, experts have regarded gene therapy as one of the most essential approaches to treating illnesses such as cancer and a variety of genetic disorders (1). Viruses are the most prevalent mechanism to deliver genes to mammalian cells. Despite several efforts to disarm viruses from causing mutations in normal tissue, there is always the potential of these viruses causing mutations(2). Many attempts have been undertaken in recent years to develop a novel approach for delivering genes to sick cells and tissues with optimum efficiency and little danger. With the advent of nanotechnology and the emergence of nanoscale materials with unique properties, promising solutions have been provided to researchers (3). By neutralizing DNA's negative charge and compressing it to smaller dimensions, cationic polymers like polyethyleneimine (PEI) improve gene transfer efficiency. Polycationions, on the other hand, limit DNA damage by destructive enzymes by triggering a process known as the proton sponge effect, which is sensitive to acidic circumstances in destructive and acidic environments of the endolysosome (4). However, despite the high advantages of these polymers, their toxicity to natural cells due to adverse interactions with cell membranes has limited the use of these polymers(5). One of the most extensively explored strategies to minimize polycation toxicity is to wrap them with biocompatibility polymers such as polyethylene glycol (PEG). Coating of nanoparticles using biocompatible polymers such as PEG has been a successful strategy to reduce the toxicity of nanoparticles, such bonds between cationic polymers and polyethylene glycol interfere with DNA release from nanoparticles (6). The contact between the PEG chain and DNA slows DNA release, causing DNA to be damaged since it does not have enough time to escape the endolysosome's unfavorable circumstances (7). Chitin compounds are mostly extracted from the skin of some marine crustaceans such as shrimp and crabs, which due to their unique properties and good biocompatibility, have been widely used in medicinal and health compounds. Chitosan is a biocompatible molecule with extremely low toxicity that has a great capacity to neutralize the negative charge of DNA and compress it owing to the presence of amine groups on its surface (8, 9).

Other nanoconductors of interest for application in drug delivery systems include magnetic nanoparticles. The superparamagnetic, supersaturating and magnetic susceptibility qualities of these nanoparticles are obtained from their intrinsic magnetic properties. Among magnetic nanoparticles, iron-cobalt nanoparticles have very high superparamagnetic properties. The use of magnetic nanoparticles in drug delivery systems, in addition to making it possible to target nanoparticles in drug delivery by applying a magnetic field, creates a good contrast in MRI imaging (10, 11). Targeting using magnetic nanoparticles reduces the side effects of the drug due to its non-specific uptake into normal cells. On the other hand, the increase in the dose of the drug in the affected area, increases the effect of the drug compared to the free drug. Due to their strong magnetic characteristics and other qualities such as temperature sensitivity and excellent biocompatibility, these nanoparticles have piqued the interest of many researchers in recent years. Various processes are used to make FeCo nanoparticles, including sol-gel, electrochemical deposition, magnetron spectromagnetic, thermal decomposition, and chemical vapor deposition (12, 13). However, these methods have limitations such as complex synthesis processes, use of environmentally hazardous materials during synthesis, as well as high cost of synthesis. The polyol reduction method is one of the techniques for the synthesis of FeCo nanoparticles, which in addition to the biocompatibility of the synthesis process, is simpler and less expensive compared to other methods for the synthesis of FeCo nanoparticles (13). Furthermore, the polyol approach prevents metal particles in the solution from oxidizing. According to the foregoing, the goal of this work is to employ the polyol approach to make FeCo nanoparticles and then combine them with chitosan to transfer the gene to MCF-7 cells. The capacity of FeCo-Chitosan nanoparticles for gene transport into MCF-7 cells was explored in this study.

Material and methods

Plasmid extraction

The extraction was carried out using the miniprep procedure as follows: In liquid LB medium, a bacterial colony with 50 mg/L ampicillin was grown overnight and incubated in a shaker incubator at 37°Cat 110 rpm. Transfer the bacterial culture to 1.5 mL microtubes and centrifuge for 5 minutes at 4000 rpm. The bacteria is suspended in 100 of solution I by vortexing and maintained at room temperature for 5 minutes after the supernatant has been removed completely (Table 1). Following that, 200 of newly produced solution II is poured into the microtubes, and the tubes are gently inverted several times before being put on ice for 5 minutes (Table 2). The microtubes are then filled with of 3 M cold potassium acetate solution (pH = 5.5) and stored on ice for 5-10 minutes after gently swirling several times. At 4°C, the microtubes are centrifuged at 13,000 rpm for 15 minutes. At this point, the supernatant is transferred to fresh tubes, and cold absolute ethanol (-20°C) is added to the microtubes in a quantity equal to the supernatant, and the tubes are held at -20 °C for 30 minutes by stirring. The tubes are then centrifuged for 15 minutes at 13,000 rpm. The top portion of the solution is removed; the DNA precipitate is washed with 70% ethanol, dried at laboratory temperature, then dissolved in of sterile distilled water, and the extracted plasmid DNA is kept at -20°C for future use.

Preparation of E.Coli susceptible cells

The following procedures were used to make transgenic susceptible cells: A single colony of E. coli strain DH5 without plasmid was grown overnight in 10 ml of antibiotic-free LB medium at 37 °C in an incubator shaker at 110 rpm. Then, in a 100 ml Erlenmeyer, 1 ml of overnight culture is added to 50 ml of new LB medium and shaken in an incubator until the OD reaches 600, which is equivalent to 0.4-0.6 under the aforementioned circumstances of the bacteria are transferred to a 50 ml Falcon and put on ice for 20 minutes. At 4 °C, the solution is centrifuged for 5 minutes at 4000 rpm. The supernatant is removed at this point, and the bacterial precipitate generated in Falcon is suspended in 20 ml of cold 50 mM CaCl2 solution and chilled for 20 minutes. The produced bacterial suspension is centrifuged and the supernatant is withdrawn after the preceding stages. The bacterial precipitate is dissolved in 4 ml of 100 mM cold CaCl2 solution at this point. Finally, the susceptible cells are transferred to 1.5 mL tubes and chilled for 30 minutes. 20 microliters of susceptible bacteria are put to each microtube.

Transgenic E.coli susceptible cells

Susceptible cell transgenics were carried out as follows: To tubes containing of sensitive cells, 40 ng of plasmid DNA or binding product was introduced and stirred gently before being kept on ice for 30 minutes. In this section, the tubes are heated to 42°C for 45 seconds before being put on ice for 10 minutes. Each tube received of antibiotic-free LB medium and was shaken at 120 rpm for 2 to 4 hours at 37 °C in an incubator shaker. The transgenic bacteria were then disseminated over a solid LB medium with ampicillin antibiotic and cultured at 37 °C for 24 hours until bacterial colonies appeared. The colonies formed on the ampicillin solid medium were obtained from transgenic bacterial cells since the plasmid utilized had the ampicillin resistance gene.

Table 1. Solution I plasmid extraction buffer

Final concentration (mM)	pH	stock(M)	material
25	8	1	Tris
10	8	0/5	EDTA
50	-	-	Glucose

Table 2. Solution II (lysis buffer) plasmid extraction buffer

100 ml Solution	Final concentration (M)	stock(M)	material
20	0/4	1	NaOH
5	0/02	20%	SDS
75	-	-	Distilled water

Synthesis of FeCo nanoparticles

A 7 to 3 molar ratio of Fe to Co, 6 g of CTAB, 60 g of betanol, and 15 g of Isoocane were placed into a container and stirred for 5 hours with a stirrer for the synthesis of FeCo nanoparticles. In 20 ml of deionized water, 2 g of cobalt acetate and 4 g of iron chloride were combined and put into the first container. Under nitrogen gas, the resultant solution was added dropwise to the initial solution. The hue of the solution has changed to black at this point owing to the reaction. A black precipitate was noticed in the solution after 5 hours of reaction at room temperature, which was collected using a magnetic magnet and cleaned with water and alcohol.

Synthesis of FeCo@Sio2-SS-COOH nanoparticles

One gram of SulfydrylatedFeCo was dissolved in 6 ml of methanol for this experiment. To alter the disulfide-thiol, 100 mg carboxyethyl 2-pyridyl disulfide was added. The nanoparticles were cleaned five times with ethanol and distilled water after a 36-hour reaction. They were then dried at room temperature in a vacuum incubator.

Synthesis of FeCo@Sio2-SS-Chitosan nanoparticles

To 8 ml of phosphate buffer at pH = 7.4, 100 mg of SS-COOH-FeCo@Sio2nanoparticles were introduced first, followed by 100 mg of EDC and 50 mg of NHS. After activating the carboxylic group for 30 minutes at room temperature, 2 ml of PBS containing 500 mg of chitosan was added and mixed for 48 hours at room temperature. Finally, the nanoparticles were rinsed in ethanol and sterile water before being dried at room temperature in a vacuum incubator.

Preparation of Chitosan-FeCo / DNA complex

The efficacy of Chitosan-FeCo nanoparticles to neutralize negative DNA charge was tested using agarose gel electrophoresis.

Electrophoresis of PCR products

The impact of Chitosan-FeCO nanoparticles on neutralizing the negative charge of DNA and protecting it against shear enzymes and ultrasonic waves was investigated using agarose gel electrophoresis. In 100 cc of TBE buffer, 0.8 g of agarose gel was added and boiled until fully dissolved. The resultant solution was stained with ethidium bromide before being placed into a tank containing the requisite wells for DNA loading. After coloring the wells with dye solution, DNA samples were transferred to PCR tubes. At 100 volts, electrophoresis was carried out for 1 hour. Finally, using a UV instrument, an agarose gel was viewed and photographed.

Evaluation of the potential of Chitosan-Fe Co nanoparticles to protect DNA against damage induced by ultrasonic and enzymatic digestion

100 µl of Chitosan-FeCo complex (containing 5 µgpDNA) with digestion buffer (50 mMTris – HCl with pH = 7.6 and 10 mM MgCL2) was treated with DNas1 enzyme (0.2 U / mg DNA) and stored in Ben Marie for 60 minutes at 37 °C. Then, to inactivate the enzyme, 5 µl of 0.5 M EDTA was mixed and maintained at room temperature for 10 minutes. To liberate pDNA from Chitosan-FeCO nanoparticles after enzymatic treatment, the Chitosan-FeCo / DNA complex was washed with TE buffer and the following suspension in 100 µl TE buffer was maintained for 24 hours at 37°C. The samples are then centrifuged (13000 rpm for 30 minutes at 4 °C) and after separating the supernatant, the DNA is precipitated with alcohol. after drying at room temperature, diluted in 30 µl of TE buffer, coupled with DNA Control (untreated with DNas1 enzyme) and DNA treated with DNas1 (0.1U / µg DNA, for 60 minutes at 37 °C), on 0.8 percent agarose gel for 2 hours in 80V Were electrophoresed. Also, to assess the impact of protection of Chitosan-FeCo nanoparticles from DNA against damage induced by ultrasound (Germany Digitec, SonorexBandelin), 100 µl of Chitosan-FeCo nanoparticles and DNA (containing 5 µg of DNA) were treated with ultrasound for 20 minutes, respectively. After separating Chitosan-FeCo nanoparticles from DNA, its supernatant is separated and the DNA of each treatment was precipitated with alcohol and after drying at room temperature, they were dissolved in 30 µl of TE buffer and each of the treatments was electrophoresed in 0.8 percent agarose gel for 2 hours in V80.

Toxicity assessment of Chitosan-FeCo nanoparticles

The MTT test was utilized for this purpose. Each well of the 96-well plates received 0.2 ml of CMF-7 cells (about 8000 cells). After 24 hours, FeCO, Chitosan-FeCo, and Chitosan nanoparticles were introduced at various quan-

tities (0.01, 0.05, 0.1, 0.25, and 0.5 mg / ml). The mixture was then held at 37 °C and 5% CO2 for 24 hours. Then, after 4 hours, MTT solution produced in PBS buffer (at a concentration of 5 mg/ml) was added to each well at a final concentration of 0.5 mg/ml MTT solution. The supernatant was removed, and the Formazan crystals were dissolved in each well with 200 L of dimethyl sulfoxide (DMSO). The plates were then incubated at 37 °C for 30 minutes while the absorbance of samples was measured at 570 nm .

MCF-7 cell transformation using Chitosan-FeCo / DNA nanoparticles

MCF-7 cells were grown in RPMI 1640 culture media with 10% FBS and antibiotics for 4 hours before transfection, and 500 l of the culture medium was transferred to 24-well plates. The supernatant culture medium was then mixed with 500 l of RPMI 1640 medium without FBS Chitosan-FeCo / DNA nanoparticles made in various Fe-Co-Chitosan to DNA ratios (100, 200, 400 g of FeCo-Chitosan to 5 g DNA) and incubated for 48 hours at 5 percent CO2 and 37 °C. Flow cytometry was utilized to identify transfected cells after the cells were seen using fluorescent microscopy (three repeats were used for each treatment).

Statistical analysis

SPSS Statistics 16.0 was used to conduct statistical analysis (SPSS Inc., Chicago, Illinois, USA). All data were evaluated using one-way analysis of variance (ANOVA) and the Tukey-post hoc test to establish statistical significance. Every piece of information is given as a mean with a standard deviation (SD). Statistically, a P value of less than 0.05 was judged significant.

Results

Confirmation of Chitosan-FeCo nanoparticle synthesis using FTIR spectroscopy

FTIR spectroscopy was used to confirm the synthesis of Chitosan-FeCo nanoparticles. The results of FTIR spectroscopy of chitosan are shown in Figure1. In Chitosan, the peaks in the range 361 to 3291 belong to the N-H and O-H groups. The C-H bond is connected to the peak recorded in the range of 2921 and 2877, and the C = O bond is related to the peak observed in the range of 1645 in the first kind of amide in chitosan. In addition, the peak in the 1325 range was linked to the C-N bond of the third type of amine in chitosan. After binding of FeCo nanoparticles to chitosan, a new peak was observed in the range of 520, which is related to Fe group bonding. These results showed that FeCo nanoparticles were successfully bonded to chitosan (Figure 1).

Plasmid transformation and extraction

To produce and propagate the plasmid DNA utilized in this investigation, pEGFP-N1 was first cryopreserved and then thawed before being extracted using the miniprepe technique. The results of agarose gel from DNA extract-

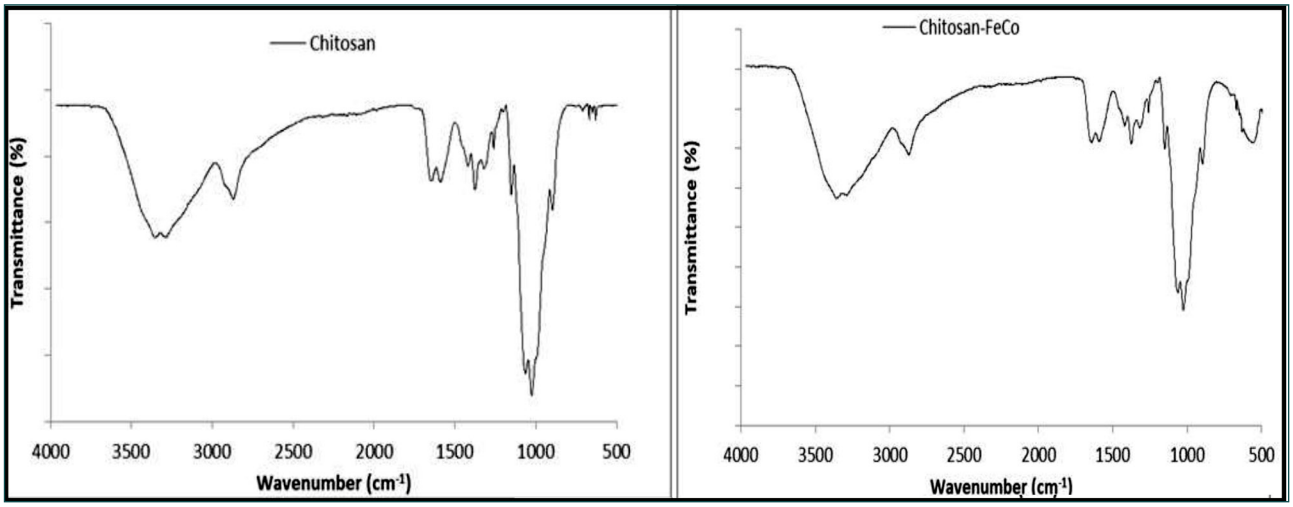

Figure.1. FTIR spectroscopy of Chitosan and Chitosan-FeCo nanoparticles.

ed from bacteria showed a suitable banding pattern of plasmid DNA. After cutting the DNA using the EcoR I enzyme, a band of 5.6 kb was visible on the agarose gel. According to the pEGFP-N1 vector map, it was expected that after cutting the pEGFP-N1 vector using EcoR I enzyme, DNA with a size of about 5.6 kb would be visible on the gel, and the results of agarose gel were consistent with the expected results (Figure 2A).

Characterization of Chitosan-FeCo and Chitosan-FeCo / DNA

The morphological characteristics of Chitosan-FeCo / DNA nanoparticles were evaluated using transmission electron microscopy. The scans revealed that the Chitosan-FeCo/DNA nanoparticles were spherical in shape and had an excellent dispersion (Figure 2B). Furthermore, the findings of the DLS device revealed that when the ratio of Chitosan-FeCo nanoparticles to DNA increased, the surface charge of nanoparticles rose. The surface charge of Chitosan-FeCo / DNA nanoparticles was -7.8 in the ratio of 10 Chitosan-FeCo to 5 DNAs, while increasing the ratio of Chitosan-FeCo nanoparticles from 10 to 400 raised the surface charge to 10.46. (Table 3).

These findings also revealed that the nanoparticle size was 214 nm at low ratios of Chitosan-FeCo nanoparticles to DNA. As the ratio of Chitosan-FeCo to DNA was

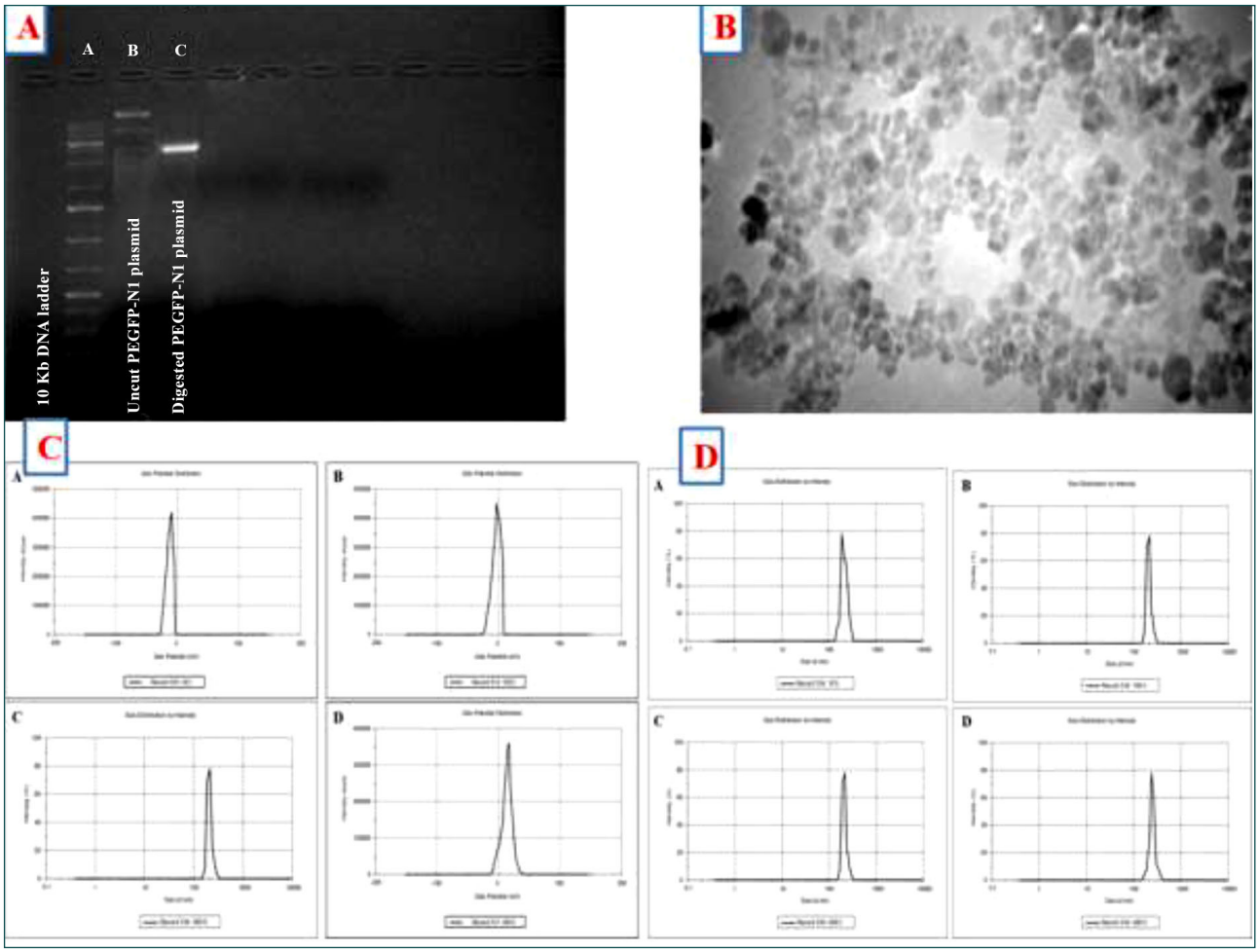

Figure.2. (Part A): Image of agarose gel from 10 Kb DNA Ladder (A), PEGFP-N1 plasmid DNA recovered from E. coli (B), and pEGFP-N1 plasmid DNA following EcoR I enzyme cleavage (Part B) (C). (Part B) Electron microscope picture of DNA-loaded FeCo-Chitosan nanoparticles. (Part C) Zeta potential of Chitosan-FeCo/DNA nanoparticles produced in various Chitosan-FeCo/DNA ratios. (Part D): Size of Chitosan-FeCo / DNA nanoparticles made in various Chitosan-FeCo/DNA ratios.

Table 3. Size and zeta potential of particles resulting from DNA complex interaction with FeCo-Chitosan nanoparticles in different weight / weight ratio (w / w%)

zeta potential	Diameter	Ratio of FeCo-Chitosan nanoparticles to DNA
1/34±-7/84	20/06±214/33	10:5
0/67±3/67-	14/17±196/33	25:5
0/32±0/24-	18/34±187	50:5
0/25±1/37	8/16±204	100:5
1/6±3/07	6/24±215	200:5
2/5±10/46	13/08±220	400:5

increased, the size of the nanoparticles shrank. The size of nanoparticles rose somewhat at a ratio of 100 to 5 Chitosan-FeCo nanoparticles to DNA and above. Due to the absence of DNA compression by the nanoparticles, the size seems to have risen in the low fraction of nanoparticles. As the number of nanoparticles increased, the DNA compression increased, so the size of the Chitosan-FeCo / DNA nanoparticles decreased. Increasing the ratio of Chitosan-FeCo to DNA nanoparticles from 100 to 5 causes excessive accumulation of Chitosan-FeCo nanoparticles around the Chitosan-FeCo / DNA complex. As a result, its size increases (Table 3) (Figures 2C and D). The results of the VSM device showed that FeCo nanoparticles had high magnetic properties (145emu/g). With a coating of FeCo nanoparticles with chitosan, the magnetic properties of these nanoparticles were significantly reduced. This value was 35 emu/g for Chitosan-FeCo nanoparticles (Figure 3A).

Investigation of the capacity of Chitosan-FeCo nanoparticles to neutralize of DNA negative charge

DNA tends to flow towards the positive charge of the electrophoresis device due to the negative charge in the phosphate group. Several studies have shown that amine groups on the surface of chitosan may neutralize DNA's negative charge (14, 15). After DNA interaction with Chitosan-FeCo nanoparticles in different ratios of Chitosan-FeCo to DNA, DNA movement was examined by agarose gel electrophoresis. As Figure 3B shows, with increasing the ratio of Chitosan-FeCo nanoparticles to DNA, DNA movement in agarose gel decreased so that in the ratio of 5: 400 Chitosan-FeCo nanoparticles to DNA, DNA movement completely stopped during agarose gel. These findings demonstrated that Chitosan-FeCo nanoparticles had a great capacity to neutralize the negative charge on DNA (Figure 3B).

The effect of Chitosan-FeCo nanoparticles on DNA protection against enzymatic digestion and ultrasound

The mammalian defense mechanism against microorganisms has DNA degradation systems. In some reports, the rate of foreign DNA destruction by the body's defense mechanism is more than 99% (16). The findings of this investigation revealed that following DNaseI enzyme treatment of DNA, the control DNA was entirely destroyed, with no bands visible in the well containing DNA uncoated with Chitosan-FeCo nanoparticles after DNase enzyme treatment. With DNA coating by Chitosan-FeCo nanoparticles, a similar effect was observed at low ratios of Chitosan-FeCo nanoparticles to DNA. However, with increasing the ratio of Chitosan-FeCo nanoparticles to DNA, the resolution of DNA bands gradually increased. These results indicate that Chitosan-FeCo nanoparticles in ratios higher than 200 Chitosan-FeCo to 5 DNAs have a good ability to protect DNA against shear enzymes (Figure 3C). Other results in this study confirm

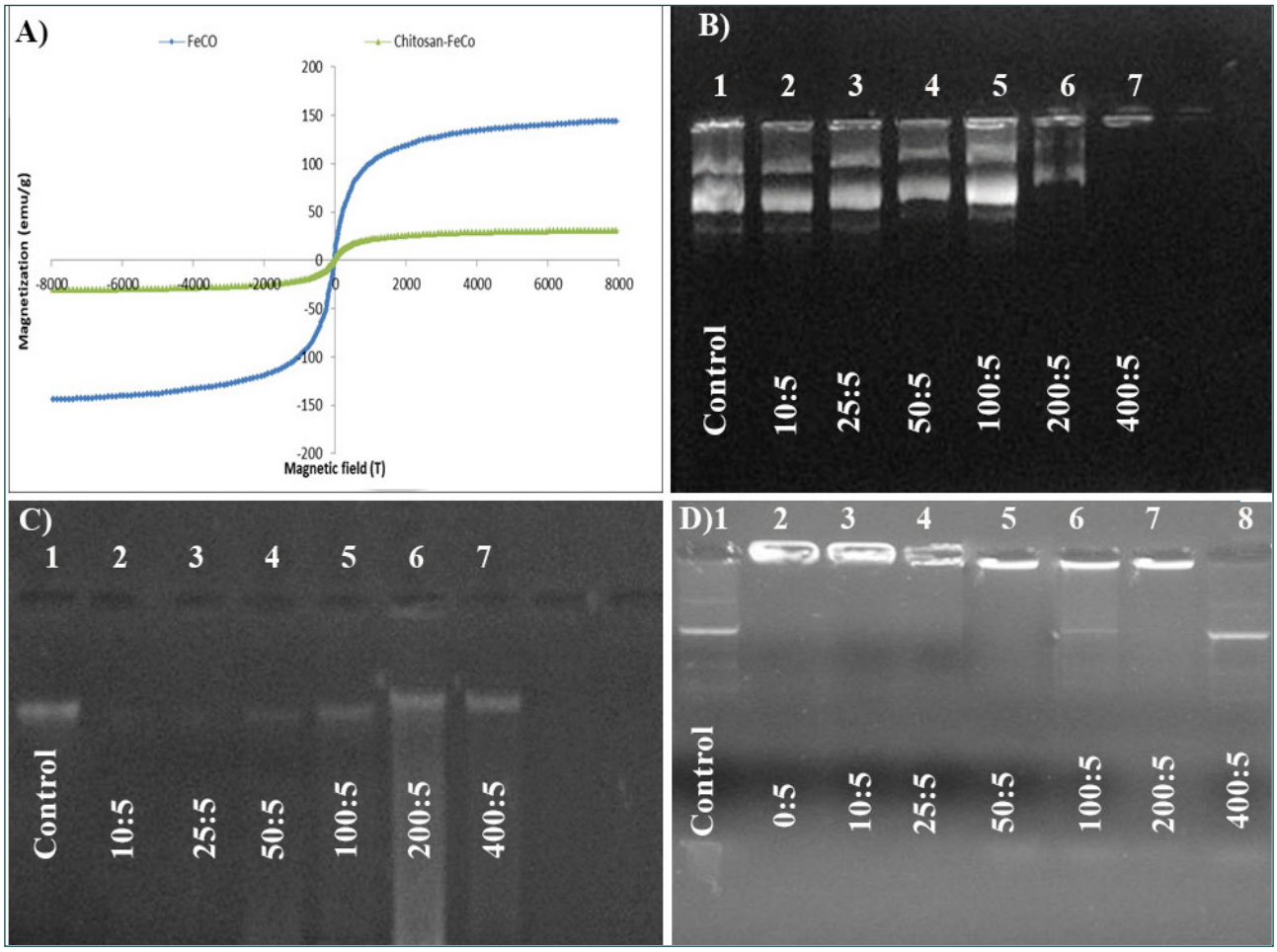

Figure.3. (Part A): VSM results of FeCo and Chitosan-FeCo nanoparticles. (Part B): Agarose gel image of the interaction between FeCo-Chitosan nanoparticles with DNA: The first to seventh wells were control DNA and DNA combined with Chitosan-FeCo nanoparticles in the ratio of 10: 5, 25: 5, 50: 5, 100: 5, 200: 5 and 400: 5, respectively. (Part C):Agarose gel Image in the protection of FeCo-Chitosan nanoparticles from DNA against DNas1 enzyme: The first to seventh wells were control DNA and combined with Chitosan-FeCo nanoparticles in the ratio of 10: 5, 25: 5, 50: 5, 100: 5, 200: 5 and 400: 5, respectively. (Part D): Agarose gel image in Protection of FeCo-Chitosan nanoparticles from DNA against ultrasound waves: The first to eighth wells were control DNA without ultrasound treatment, control DNA after treatment with ultrasound and DNA coated with Chitosan-FeCo nanoparticles in the said ratios.

the ability of Chitosan-FeCo nanoparticles to protect DNA against ultrasound-induced damage. After DNA treatment with ultrasound, the control DNA was entirely destroyed. In this work, the impact of Chitosan-FeCo nanoparticles on DNA protection against DNase I and ultrasound followed a similar pattern. In other words, by increasing the ratio of Chitosan-FeCo nanoparticles to DNA, the effect of Chitosan-FeCo nanoparticles in protecting DNA against ultrasound increased. In light of the use of ultrasound in the transfer of macromolecules such as DNA to plant cells and microorganisms, the usage of these nanoparticles seems to be efficient in improving gene transfer efficiency in these cells (Figure 3D).

Toxicity of FeCo-Chitosan nanoparticles on MCF-7 cells

The toxicity of FeCo-Chitosan nanoparticles was assessed using the MTT test on MCF-7 cells. The findings indicated that chitosan nanoparticles at a concentration of 0.5 mg/ml did not influence on the viability of MCF-7 cells. FeCo nanoparticles, on the other hand, drastically decreased the vitality of MCF-7 cells, with the lowest cell viability found in MCF-7 cells treated with 0.5 mg of FeCo nanoparticles (27 percent). The toxicity of FeCo nanoparticles was lowered by coating them with chitosan (Figure 4A).

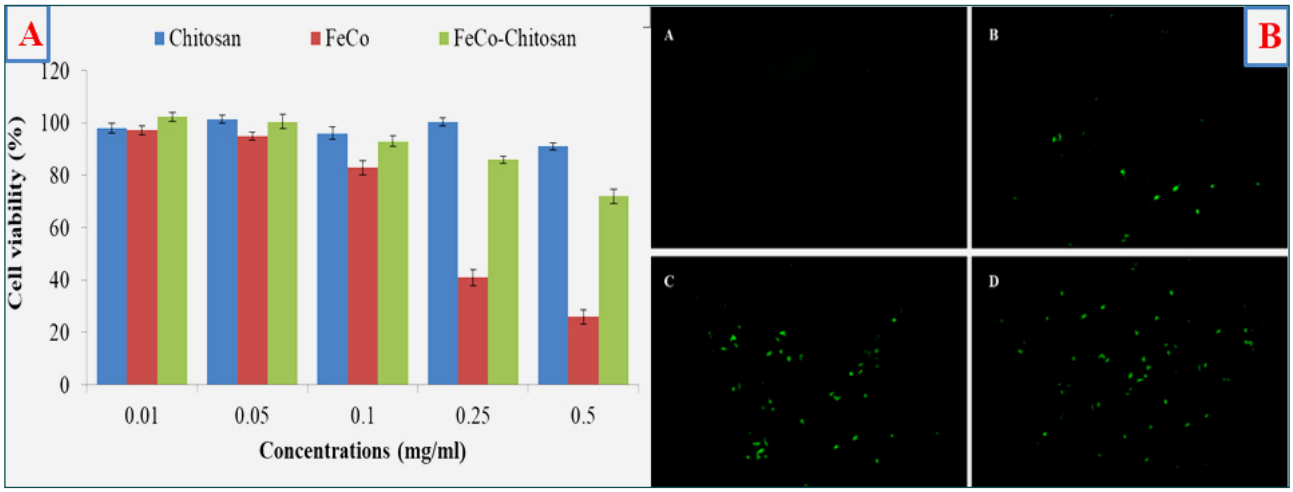

Figure.4. (Part A): Effect of different concentrations of Chitosan, FeCo and FeCo-Chitosan nanoparticles on MCF-7 cell viability. (Part B): Fluorescence microscope image of control cells (A), and cells treated with Chitosan-FeCo / DNA nanoparticles prepared in 100: 5, 200: 5 and 400: 5 ratios of Chitosan-FeCo: DNA.

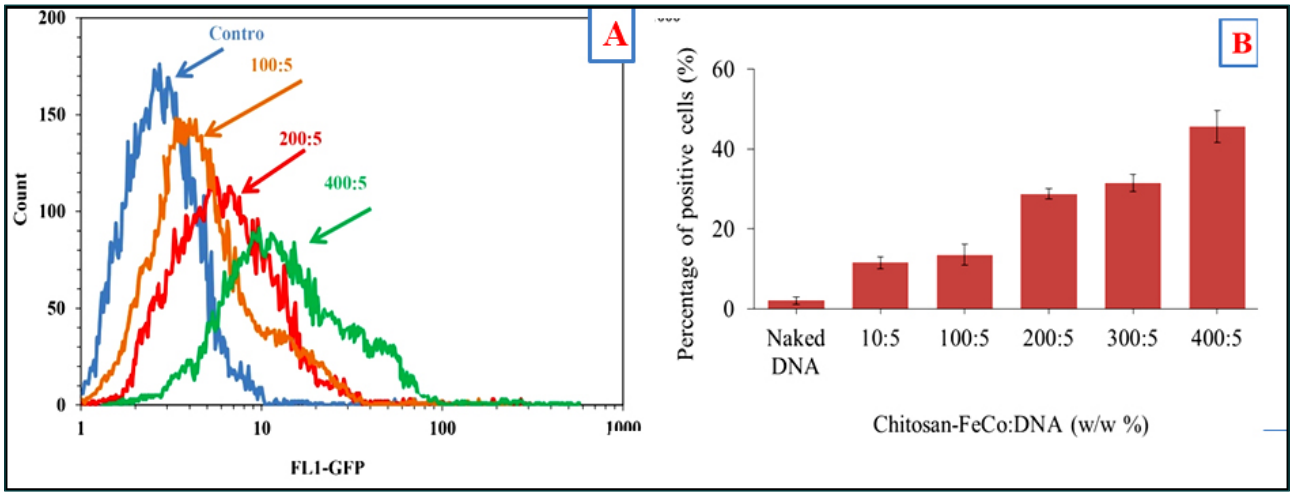

Figure.5. (Part A): Flow cytometry results from MCF-7 cell treated with Chitosan-FeCo / DNA nanoparticles prepared in different ratios of Chitosan-FeCo to DNA. (Part B): Comparison of gene transfer percentage to MCF-7 cells using Chitosan-FeCo nanoparticles.

Transfection of MCF-7 cells using Chitosan-FeCo nanoparticles

Fluorescence microscopy and flow cytometry were used to assess the potential of Chitosan-FeCo nanoparticles to transfer DNA to MCF-7 cells. The GFP gene is one of the most widely used genes to confirm the ability of gene carriers in mammalian cells. Under UV light, the green hue created by GFP protein may be seen in cells after the expression of the GFP gene. The capacity of these nanoparticles to transfer the gene was shown by imaging MCF-7 cells treated with Chitosan-FeCo/DNA nanoparticles. Flow cytometry was used to quantify the gene transfer efficiency. Increasing the ratio of Chitosan-FeCo nanoparticles to DNA increased the efficiency of gene transfer to MCF-7 cells. The highest percentage of gene transfer was observed in cells treated with Chitosan-FeCo / DNA nanoparticles prepared in the ratio of 400 Chitosan-FeCo to 5 DNAs (43%) (Figure 4B) and (Figure 5A and B).

Discussion

Previous research has been conducted in recent years to use nanoparticles to transport various kinds of pharmacological agents, such as DNA, peptides, and small-molecule medicines, into cancer cells and tumor tissues

(17, 18). Compared to other nanoparticles, Magnetic nanoparticles have become one of the most widely used nanostructures in medical applications, especially drug delivery, due to their unique advantages. Among the unique properties of these nanoparticles, we can mention the properties of superparamagnetic, supersaturation and magnetic susceptibility. These properties allow magnetic nanoparticles to be easily transferred to the target tissue in the body by applying a magnetic field. Desirable and stable biomedical properties can be created for these nanoparticles by using different surface coatings, and the particokinetic effects and toxicity of magnetic nanoparticles due to interactions with cells or biological proteins can be avoided, resulting in increased magnetic nanoparticle biocompatibility. These nanoparticles have very strong magnetic characteristics, and even after being coated with biocompatible compounds like chitosan, their magnetic activity is unaffected. The attachment of chitosan to FeCo nanoparticles dramatically decreased its magnetic characteristics, as seen in Figure. According to current research, Chitosan-FeCo nanoparticles' magnetic characteristics make them appropriate for a variety of medication administration and MRI imaging applications (19, 20).

The use of cationic nanoparticles for gene transfer is common compared to other nanoparticles due to the high gene transfer efficiency (18). These nanoparticles transport DNA into the cell by neutralizing the negative charge. In addition, DNA compression by cationic nanoparticles creates a suitable morphology and facilitates the transport of DNA-containing nanoparticles into the cell (21). While DNA is excreted by the cell membrane due to a negative surface charge (the cell membrane is negatively charged), on the other hand, due to its large size and inappropriate morphology, DNA has a low ability to transfer into cells and pass through the cell membrane (22, 23). Due to the presence of restriction enzymes in plasma as well as macrophages in the immune system, it is impossible to transfer DNA to the patient's tissue for the purpose of gene therapy and treatment of the damaged gene. Recent studies have shown that if DNA enters the cell, it is rapidly detected and destroyed by the lysosome (24, 25). Neutralization of DNA negative charge by amine groups present on the surface of chitosan has been proven in several reports (14, 15). In the present study, by increasing the ratio of Chitosan-FeCo nanoparticles to DNA, DNA movement in agarose gel decreased.In the ratio of Chitosan-FeCo nanoparticles to DNA 400 to 5, DNA movement was completely stopped during agarose gel. These findings demonstrated that Chitosan-FeCo nanoparticles had a great capacity to neutralize DNA's negative charge.

Pouponneau et al. Used FeCo nanoparticles to deliver doxorubicin to the deep tissues of rabbit liver. In this study, FeCo nanoparticles were first synthesized and then several properties such as the morphology of the resulting nanoparticles, doxorubicin release pattern from FeCo / DOX nanoparticles and its ability to deliver the drug in vivo on rabbit liver tissue were investigated. The results showed that FeCo / DOX nanoparticles have a magnetic saturation of 75 emu /g. Also, their results showed that these nanoparticles have MRI imaging properties. FeCo / DOX nanoparticles had a spherical structure and a size of about 50 μm. The research of doxorubicin transfer by these nanoparticles to rabbit liver, however, revealed that these nanoparticles had a great potential for drug transfer and MRI imaging (26). Yang et al. synthesized FeCo nanoparticles for the first time using the polyol reduction approach in another work. The results of their research showed that all nanoparticles obtained have very high magnetic properties. So that all nanoparticles obtained in this study had a surface saturation higher than 220 emu / g. The highest surface saturation in this study was 273 emu / g, which was observed in Fe55Co45 nanoparticles (26). In the present study, the results of the VSM device showed that FeCo nanoparticles had high magnetic properties (145emu/g). With coating of FeCo nanoparticles with chitosan, the magnetic properties of these nanoparticles were significantly reduced. This value was 35 emu/g for Chitosan-FeCo nanoparticles. The results of the MTT test also showed that chitosan nanoparticles up to the concentration of 0.5 mg/ml had no significant toxic effect on MCF-7 cells viability. However, FeCo na-

noparticles significantly reduced the viability of MCF-7 cells, so that the lowest cell viability was observed in the treatment of MCF-7 cells with 0.5 mg of FeCo nanoparticles (27%). Coating of FeCo nanoparticles by chitosan reduced the toxicity of FeCo nanoparticles.

The positive charge in cationic nano-vector causes interaction with cell membranes and ultimately causes gene transfer into the cell through endocytosis (27). Flow cytometry and fluorescence microscopy findings revealed that FeCo-Chitosan nanoparticles could securely transfer and release DNA to MCF-7 cells in this investigation. These findings also revealed that increasing the ratio of Chitosan-FeCo nanoparticles to DNA improves gene transfer efficiency to MCF-7 cells. Cells treated with Chitosan-FeCo / DNA nanoparticles produced in a ratio of 400 Chitosan-FeCo to 5 DNA showed the greatest percentage of gene transfer.

It is well known that DNA cleavage by restriction enzymes is performed only if the cleavage enzyme binds to its specific site on DNA. Binding of cationic nanoparticles to DNA creates a space barrier, in other words, the enzyme site is occupied by these nanoparticles. Therefore, the enzyme cannot bind to DNA and cleave it. As shown in Figure, increasing the ratio of Chitosan-FeCo nanoparticles to DNA increases the resolution of healthy DNA bands.Ultrasound is commonly used to transmit genes to wall cells, such as plant cells and microorganisms. By creating cavitation, these waves cause cavities in the surface of the cell wall that facilitate the entry of DNA into the cell. However, DNA damage by these waves is considered as one of the disadvantages of using this method in gene transfer (28). The capacity of Chitosan-FeCo nanoparticles to protect DNA from ultrasonic was shown using an agarose gel image. The compression of DNA by these nanoparticles seems to minimize the amount of DNA that comes into contact with ultrasound. In addition to boosting the effectiveness of gene transfer to walled cells, the use of nanoparticles to shield DNA from damage induced by ultrasound may improve the efficacy of gene treatment using sonotherapy (28).

Conclusion

The findings of this research demonstrated that Chitosan-FeCo nanoparticles had a high capacity to transfer genes to MCF-7 cells and also protect DNA from damage induced by enzymatic digestion and ultrasound. These nanoparticles may be employed as a good carrier for gene transfer in the targeted therapy of cancer.

Ethical Issues

In all stages of working, ethical principles were observed with the ethics code IR.UMA.REC.1399.184 in accordance under the Charter of Laboratory Ethics of MohagheghArdabili University.

Consent

All authors declared that written informed consent was obtained from both the patient and their attendant for the publication of these articles and accompanying images. However, the identification of the patients is not disclosed anywhere in the article.

Disclosure

The authors report no conflicts of interest.

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