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RESEARCH ARTICLE

Cisplatin-loaded superparamagnetic nanoparticles modified with PCL-PEG copolymers as a treatment of A_{549} lung cancer cells

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ABSTRACT

Magnetic nanoparticles have been highly regarded because of their unique properties, such as hyperthermia, medicine control release, and diagnostic applications. The role of these magnetic nanoparticles as medicine delivery carriers is bolder due to certain properties, in addition to the usual properties of nanomaterials, The main aim of the current paper is to offer a new system for the modification of Fe_3O_4 (SPIONs) superparamagnetic nanoparticles physically and chemically with polymers through physical retention. These modified nanoparticles have been used to encapsulate cisplatin as an anticancer medicine and the effect of nanocapsulated cisplatin has been studied in lung cancer (A_{549}) cell line. Using ring-opening polymerization Triblock copolymer PCL-PEG-PCL was prepared of ε-caprolactone (PCL) in the presence of polyethylene glycol (PEG). Magnetic iron nanoparticles were also prepared and identified. Using Fourier Transform Infrared Spectroscopy (FTIR), the bulk features of the copolymers were determined. Nanoparticles loaded with Cisplatin have been ready using the copolymer containing iron superparamagnetic nanoparticles via double emulsion solvent evaporation method and evaluated for medicine entrapment efficiency (%), the quantity of medicine, size, and surface morphology. Cytotoxic tests have been considered using MTT assay method in lung carcinoma (A_{549})-treated cells. As well as, using scanning electron microscopy, Fourier Transform Infrared Spectroscopy (FTIR) and X-ray powder diffraction (XRD) the particles have been specified. The results of the study in vitro showed that Fe_3O_4-PCL-PEG nanoparticles did not have a cytotoxic effect and were biocompatible. The anti-proliferative effect of cisplatin encapsulated in magnetic nanoparticles was much earlier faster than pure cisplatin and enhanced the decrease in IC50 rate significantly. The results of the study demonstrated that nanocapsulated cisplatin had a significant cytotoxic and anticancer effect in vitro of the lung cancer cell line and it can be concluded that this approach has the ability to fail some of the main chemotherapy constraints and can be an appropriate approach for future programs in the treatment of lung cancer.

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INTRODUCTION

Cancer is one of the most common diseases of death in developed and less developed countries (1). According to the American Cancer Society, in 2017, about 26% of all mortality from cancer is lung cancer (2). Even with the last advancement in cancer treatment, there is a teeny definite development in the treatment of lung cancer. Those who are most at risk of lung cancer are adults over the age of 50 years and those who have a history of smoking, or smoking or pipe and hookah (3). Although smoking is a major cause of lung cancer, people who have never smoked can also be exposed to lung cancer for reasons such as past pulmonary infections, and environmental and genetic factors. Those who do not smoke include 15% of cases of lung cancer. These are commonly found in exposure to asbestos, radioactive gas, genetic elements and air pollution(4). The most communal indications are including coughing up blood shortness of

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breath and weight loss(5). Typically, signs of lung cancer do not appear as long as the disease is in its advanced stages. Usually, signs of lung cancer do not appear as long as the disease is in its advanced stages, which is why lung cancer is not usually diagnosed at an early stage (6). The 5-year survival rate for valetudinarians with lung cancer is 16%, as most patients are diagnosed with advanced stage (7). The first diagnostic method for lung cancer is the chest radiograph. Chest radiography is able to show the presence of the tumor, lung collapse, widening of the mediastinum, inflammation of the pulmonary etc. In addition, CT scans are used to determine the type and stage of cancer (8). Lung carcinomas are classified as Small Cell Carcinoma (SCLC) and Non-Small Cell Carcinoma (NSCLC) (for example adenocarcinoma, squamous cell carcinoma, and large cell carcinoma)(9). Common treatments for lung cancer include surgery, chemotherapy, and radiation therapy. In the chemotherapy of lung cancer, platinum-based medicine (Cisplatin or Carboplatin) and Gentamicin, Paclitaxel, Docetaxel, etc. are commonly used (10). The common chemotherapy for SCLC is cisplatin or combination of Doxorubicin, Vincristine, and Cyclophosphamide(11, 12). Chemotherapy as a basic treatment for lung cancer has poor results due to medicine resistance, so it is very important to make changes to these therapies using modern methods (13) Since the 1990s, new medicines have been developed and proposed for the treatment of lung cancer, yet cisplatin is still a major medicine for the treatment of lung cancer(14). Nanotechnology has been considered with the use of nanoparticles and the development of targeted medicine delivery methods for the diagnosis and treatment of diseases. The use of nanocarriers for medicine delivery to cancerous tissues will reduce side effects and eliminate medicine resistance as well as further medicine sustainability(15). Today nanoparticles are widely used in medical and diagnostic studies. One of the nanoparticles that can be used to promote targeted medicine delivery to cancer cells is polycaprolactone (PCL), but there is a problem with the application of this polymer because the lack of electrical charge on the surface of these particles leads to be swallowed by Phagocytes (16). They also degrade very slowly because of hydrophobicity. Therefore, polyethyleneglycol (PEG) is used to overcome these problems. Poly ethylene glycol has a hydrophilic property and has a dramatic effect on the reduction of trapping by the body's Reticulum Endothelial System (RES) and increased solubility(17, 18). Recent developments in nanotechnology have advanced ability to modify the structures and properties of Magnetic nanoparticles (MNPs) for biomedical applications. Moreover, Magnetic nanoparticles have a high potential in the recognition and treat the diseases, especially cancer. The use of these nanoparticles as contrast enhancing agents in the conventional method of magnetic resonance imaging and also as a nanocarriers in modern medicine delivery systems have been of interest to researchers over the past few years. Among the iron oxide nanoparticles, magnetic is the only Nanoparticle that approved for clinical use by the Food and Medicine Administration (FDA) (18-20). Magnetic nanoparticles are mainly used in therapeutic systems and diagnostic applications such as imaging, sensors, targeting, medicine delivery, hyperthermia, chemotherapy etc. (Fig. 1)

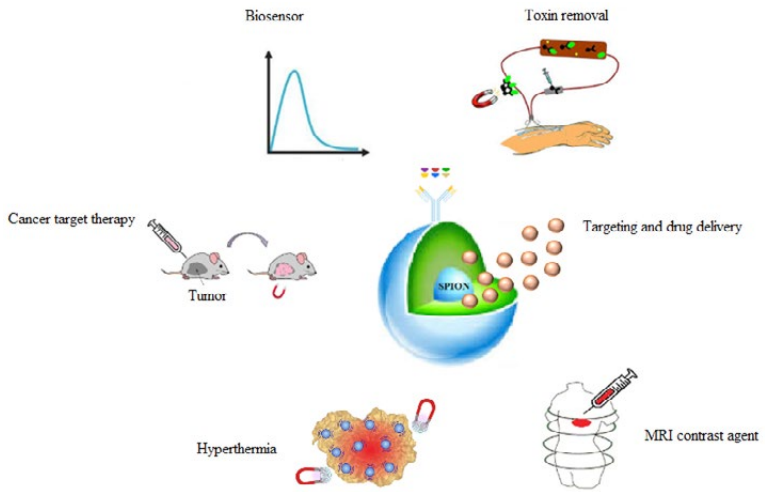

Fig. 1: Superparamagnetic iron oxide nanoparticles (SPION) applications

(19). Physical and chemical properties and appropriate size of these particles have made them suitable as contrast agents for use in cellular and molecular imaging of cardiovascular disease and MRI. In addition, using the induction excitation of biocompatible superparamagnetic nanoparticles and the fluctuations of magnetic moments within the nanoparticles can generate additional heat (41-47 °C) in the tumor area and thereby destroy the cancer cells, while not damaging healthy cells. As magnetic nanoparticles react to the external field, they are therefore have received much attention in the field of active targeting and drug delivery.

MNPs are a major category of nanoparticles with the capability to develop common clinical therapy and diagnostic procedures. While primary studies in this regard can be traced back to some decades, a new round of attention to nano-technology has meaningfully extended the scope and depth of MNPs studies (20). The encapsulation of anti-cancer medicines in triblock polymers that are biodegradable poly-(ethylene glycol)-poly (e-caprolactone)-poly (ethylene glycol) (PEG-PCL-PEG) nano-particles might show benefits over other delivery systems, such as liposomes (Fig. 2). Some of these benefits are well known in previous studies , for example, many of very hydrophobic to highly hydrophilic medicines can be encapsulated in PCL-PEG-PCL nanoparticles, the release rate can be specific to the size and loading and modification (21, 22). PEG is hydrophilic non-immunogenic and non-toxic. Since PCL is a biocompatible, biodegradable, semi-crystalline polymer with a low glass transition temperature, so the PEG-PCL nanoparticles are biocompatible and biodegradable that act as a micelle. The outer layer is made up of hydrophilic PEG and has furtiveness properties so (RES) cannot detect it. They have a longer circulation time in the bloodstream and more enhanced permeation and retention (EPR) effects, which allows them to enter into the solid tumors. (23, 24).

Cisplatin or Cis-Platinum or Cis-Diamine Di-Chloro-Platinum (II) (CDDP) is a platinum-grounded chemotherapy medicine used to treat various types of cancers including sarcoma, some carcinomas (such as small-cell lung cancer and ovarian cancer), Lymphomas and germ cell tumors are used(26, 27). While these agents have side effects that limit the dose level. Side effects of cisplatin embrace nausea, vomiting, peripheral neuropathy, ear toxicity, liver toxicity, increased blood urea, encephalopathy, nephrotoxicity, etc. (28). Due to the constraints in dosing cisplatin, several clinical prosecutions have been or being indicated to better lung cancer products using cisplatin in combining with other elements like gemcitabine 5-fluorouracil and purposeful treatments making small-molecule suppressors or monoclonal antibodies (29, 30). Other methods to improve cisplatin performance in vivo examined. Nano-particles and local delivery of medicine methods like chemotherapy-filled films, and gels are establishing to advance medicine approval along minimizing systemic complications (31). Indeed, nanoparticles loaded with cisplatin have been estimated in many clinical prosecutions coupled with favorable outcomes and other cisplatin medicine delivery materials like those that films, adhesives, and gels armed for topical prescription are becoming important in coping with lung-related thoracic cancers (32-36). In this report, we produce triblock poly (ethylene glycol)-poly (e-caprolactone)-poly (ethylene glycol) nanoparticles and have studied clinical value of them for the safe and performative transfer of the Cisplatin as an anti-cancer agent. Decreasing the nephrotoxicity induced by cisplatin is highly appropriate for curing lung cancer. Nano-technology offers a strong device for reducing the overall absorption of cisplatin in the kidneys with the absorption fraction of cisplatin among the kidneys and blood. So, new therapeutic methods and uncommon preparations of Cisplatin are to-the-point desired to advance the rate of handling in the patients with lung cancer a meager diagnosis.

PRACTICAL STEPS AND MATERIALS

Ferric chloride hexahydrate ($FeCl_3.6H_2O$), ferrous chloride tetrahydrate ($FeCl_2.4H_2O$), and ammonium hydroxide (25 wt %) from Fluka (Buchs,

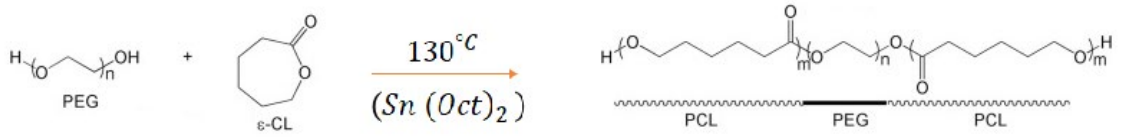

Fig. 2: Scheme of the synthesis of the PCL-PEG-PCL copolymer(25)

Switzerland), D, L-lactide, glycolide, dimethyl sulfoxide and Poly (ethylene glycol) (M_w=4000) from Sigma-Aldrich (St Louis, MO), Acryloyl chloride, stannous octoate ($Sn(Oct)_2$), □-caprolactone (□-CL) (Mw=14000) from Sigma-Aldrich (St Louis, MO, USA) and Cisplatin from Merck have been purchased. Human lung carcinoma cell line ($A549$) was purchased from Pasteur Institute of Iran, Tehran, Iran. X-ray diffraction, Rigaku D/MAX-2400 x-ray diffractometer with Ni-filtered Cu Kα radiation, and scanning electron microscopy (SEM) measurements were conducted using VEGA/TESCAN. The capacity to load medicine and its free behavior were specified with an ultra-violet visible 2550 spectrometer (Shimadzu, Tokyo, Japan). Infrared spectra were registered in real-time with a Perkin Elmer series FTIR. The magnetic feature was measured using magnetometer on a vibrating sample (Meghnatis Daghigh Kavir Co, Kashan, Iran) at room temperature. The average molecular weight was attained using gel permeation chromatography done in dichloromethane (CH_2Cl_2) with a Waters Associated Model ALC/ gel permeation chromatography 244 device. The specimens have been homogenated with a homogenizer (Silent Crusher M, Heidolph Instruments GmbH, Schwabach, Germany). The organic phase has been evaporated by the rotary (Rotary Evaporators, Heidolph Instruments, and Hei-VAPseries).

Conditions for culturing of cancerous cell lines

The A_{549} cell line after distinguishing IC50 (1*10) cells have been treated with different concentrations of PCL-PEG-Cisplatin. For the control group, the same volume of 10% dimethyl sulfoxide without -PCL-PEG-Cisplatin have been added to the flask. Flasks were incubated at 37 containing 5% with an incubator having humidified atmosphere for 24-h exposure duration.

The synthesis method of Superparamagnetic nano-particles

Superparamagnetic nanoparticles have been synthesized by the help of an improved chemical co-precipitation procedure (37).

Based on this procedure 7.5684 g the exact value of $FeCl_3.6H_2O$ (0.028 mol) and 3.1736 g of $FeCl_2.4H_2O$ (0.016 mol), have been dissolved in 320mL of deionized water (30 minutes deoxygenation), resembling Fe^{2+}/Fe^{3+} = 1/1.75. Then the mixture has been stirred under nitrogen at $80^{\circ C}$ for 1 hour after a few minutes, 40 mL NH_3H_2O have been added rapidly into the mixture, stirred under nitrogen for another hour (pH between 9.5 and 11). As soon as the NH_3H_2O is added, the yellow solution turns black which indicating the formation of magnetic nanoparticles. And then the solution cooled to room temperature. The resulting particles were washed several times and separated by the magnet. Finally, the magnetic nanoparticles were dried under vacuum at $70^{\circ C}$ (Fig. 3).

Preparation of PCL-PEG triblock copolymer

PCI-PEG--PCL triblock copolymer has been synthesized using PCL initiated PEG whit ring-opening polymerization method. A certain amount of PEG and PCL monomers with a ratio of 3 to 1 in a three-neck Round-Bottom Flask and nitrogen Presence in a bath of silicone oil on stirrer equipped with a heater, up to 180 ° C for 5 minutes, melted. Balloons on a heater with a magnetic stirrer were heated. For precise temperature control at all stages of a thermometer in the bath with constant and equal height from the bottom of the balloon was placed. After melting of the PEG, the temperature was increased 130. Then octoate molten tin catalyst (0/05% by weight of raw material) was added as a catalyst to start the Polymerization reaction polymerization at this temperature with gentle stirring and continued nitrogen gas flow. (In the

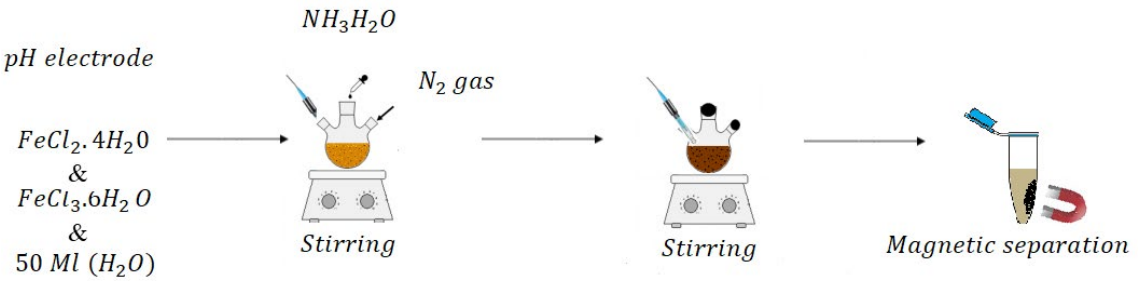

Fig. 3: Schematic of superparamagnetic nanoparticles synthesis

early hours of Polymerization should completely prevent to entry oxygen gas into the balloon). After Polymerization, the solution was cooled to room temperature. Gross Polymer for purification and isolation of residual monomers, solid Polymer was dissolved in dichloromethane and was poured into a large volume of diethyl ether and dry. Diethyl ether as a solvent action and lead to the deposition polymer. This process was repeated twice. Polymer smoothing method of the solvent in a vacuum attached to the desiccating.

Preparing cisplatin-encapsulated Fe_3O_4 magnetic nano-particles modified using PCL-PEG copolymer

For the preparation of magnetic nano-particles PCL-PEG-loaded with cisplatin, double emulsion technique was used. 5mg of magnetic nanoparticles and 30mg cisplatin in 5 ml dichloromethane in which 300mg of PCL-PEG copolymer solved. Emulsion with sonicator probe with the power of 10W for 45 seconds have been sonicated. As a result, the primary emulsion W/O was prepared. Original emulsion stabilizers containing an aqueous solution (solution 1% PVA) with a volume of 10.6 ml was added and again with sonicator probe was homogenized in 18W for 60 seconds. Double emulsion W/O/W to obtain the highly agitated at room temperature so that the organic phase dichloromethane absolutely mixed (cause evaporation at the end) to increase medicine encapsulation, the suspension was lyophilized (Fig. 4). The content and the efficacy of encapsulation of Cisplatin in nano-particles corrected with PCL-PEG copolymer were disintegration nanoparticles in dichloromethane. The Cisplatin condensation was specified by spectrophotometry at 210-280nm. The efficacy of medicine encapsulation was measured using the equations below: (DEE%: Medicine Encapsulation Efficacy Percent) (38).

$$\%DEE = \left(\frac{Amount\ of\ drug\ entrapped}{Amount\ t\ of\ drug\ added}\right)\times 100$$

$$Drug\ content\ (\%) = \left(\frac{Amount\ of\ entrapped}{Amount\ t\ of\ nanoparticles}\right)\times 100$$

Characterization of nanoparticle morphology using SEM

The morphology of the magnetic nanoparticles and Cisplatin-encapsulated magnetic nanoparticles modified with PCL-PEG copolymer nanoparticles was observed using scanning electron microscopy (SEM) as an accelerating voltage of 10.0 kV (VEGA/TESCAN) and samples were coated with gold. The diameter of nanoparticles was determined by ImageJ software (20 nanoparticles were selected at random).

The study of in vitro medicine release

Examining the releasing of synthesized cisplatin encapsulated in nano-particles corrected with PCL-PEG copolymer, 3mg of medicine-Encapsulated nanoparticles were scattered in 30mL of phosphate-buffered solution (pH=7.4). The specimens were incubated at $37^{\circ C}$. At the dedicated time intervals, a 3mL specimen was taken out and the same volume was constituted again with the

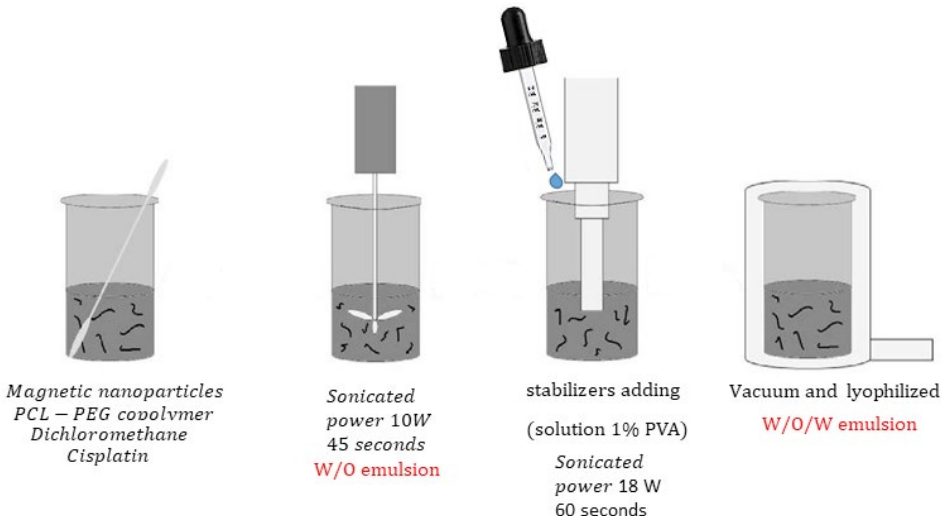

Fig. 4: schematic of cisplatin-encapsulated Fe_3O_4 magnetic nano-particles modified using PCL-PEG copolymer

addition of 3mL of a fresh phosphate-buffered solution and acetate buffer to each specimen. After the test, the samples were analyzed with the help of ultraviolet spectrofluorometry to specify the value of Cisplatin released (210 nm and 265nm for Cisplatin measurement).

Cytotoxicity assays

The A_{549} lung cancer cell line was cultured in a RPMI-1640 (Gibco, Invitrogen, Carlsbad, CA) culture medium containing 10% Fetal Bovine Serum (FBS)(Gibco, Invitrogen, UK), 0.05 mg/mL penicillin G (Serva Co, Germany), 2mg/mL sodium bicarbonate,0.08 mg/mL streptomycin (Merck Co, Darmstadt, Germany), and incubated at $37^{\circ C}$ and 5% humidity in sterile flasks. After several days the cell count reached 10^6 per ml. as soon as a sufficient number of cells were obtained the cytotoxic effect was studied at 24, 48, and 72 h using MTT assays. In short, 1000 cells/well were cultivated in a 96-well plate. at first 20 µl of the suspension containing 10 cells was added to each well from a 96-well plate, after 24 hours, 200 µl of the various dilutions prepared with the culture medium added to each well (each dilution was triple Used). Wells that only contained a cell-free medium were used as a blank of Elisa Reader. Cell-free wells without treat as controls, wells containing cells treated with Fe_3O_4-PCL-PEG-Cisplatin and wells containing treated cells with pure cisplatin were used. Different treatment times of 24, 48 and 72 hours for treatment of cells with different dilutions of the medicine were considered. After the treatment was complete, 200 µl of fresh medium and 50 µl of MTT solution were poured into each well. Finally, the environment of each well was evacuated and 200 µl DMSO and 25 µl Sorenson buffer were added to each well. To dissolve the formazan crystals the plates were shaken then the amount of optical absorption was read by the ELISA reader (Bio-Tek Instruments, Winooski, VT) at 570nm with a reference wavelength of 630 nm. The following formula was used to convert OD to the percentage of live cells.

$$\left[\frac{OD_{test}}{OD_{control}}\right]\times 100$$

At the end, optical absorption was analyzed using SPSS 16 software. And IC_{50} of nanoparticles including Fe_3O_4-PCL-PEG-Cisplatin, blank and pure cisplatin were calculated at 24, 48 and 72 hours (Fig. 5).

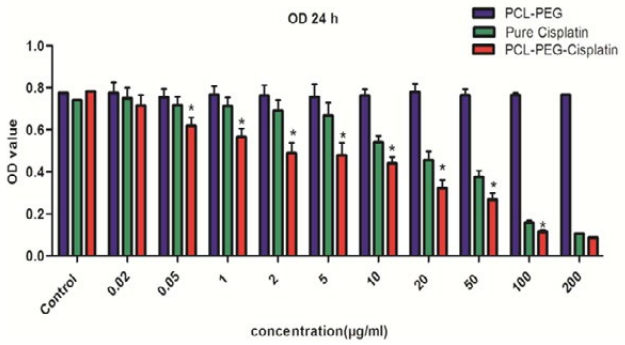

Fig. 5.a: The results of the treatment cells (A_{549}) with different doses of PCL-PEG, pure cisplatin, PCL-PEG- cisplatin in 24 hours

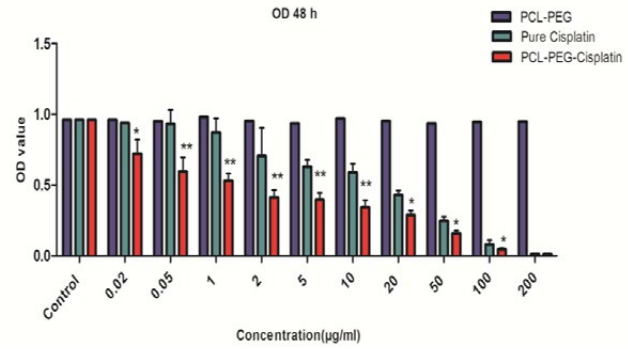

Fig. 5.b: The results of the treatment cells (A_{549}) with different doses of PCL-PEG, pure cisplatin, PCL-PEG- cisplatin in 48 hours

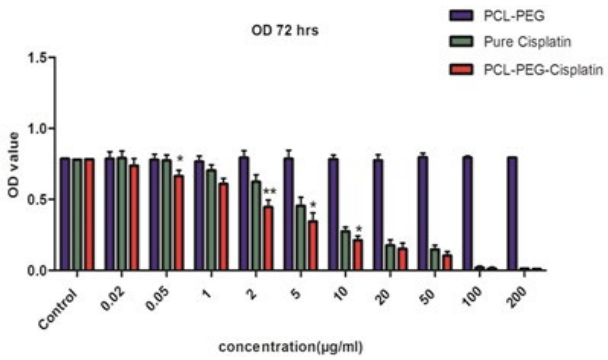

Fig. 5.c: The results of the treatment cells (A_{549}) with different doses of PCL-PEG, pure cisplatin, PCL-PEG- cisplatin in 72 hours

RESULTS AND DISCUSSION

A perfect preparation of biocompatible nano-sized particles leads to great medicine loading along sustained-release fractures, so letting medicine at a therapeutic concentration release in the targeted place and minimizing medicine inefficiency and confrontational impacts. Many methods have been investigated for this purpose(39, 40). In this study, we presented a new formulation of cisplatin that is less toxic and more effective than pure cisplatin. In this method for reducing unfavorable features of Cisplatin, we used super-paramagnetic nanoparticles modified with PCI-PEG--PCL triblock copolymer to encapsulate the Cisplatin anti-cancer medicine. These nanoparticles arranged and modified as a carrier that can be used for targeting to the wide range of solid tumors. For this purpose, the PCL-PEG-PCL triblock copolymer was synthesized by ring-opening polymerization methods using poly (ethylene glycol) and poly (Ɛ-caprolactone) monomers. It was found that The FTIR spectra were in accordance with the PCL-PEG-PCL copolymer structure(41). The molecular weight has been determined using gel penetration chromatography. In this work, nanoparticles containing cisplatin were prepared by Double emulsion W/O/W method. The percentage of medicine entrapped in nanoparticles was determined 70% and the particle size was around 50-70nm. The results showed that the cisplatin-encapsulated nanoparticles exhibition pH sensitivity and can be used for targeting extra-cellular pH and could be an effective carrier for anti-cancer medicines. It is awaited that, the cisplatin encapsulated nanoparticles can show enhanced cytotoxicity at tumor pH, compared with normal pH=7.4. The Result shows that magnetic nanoparticles could be impressive as a carrier for medicine delivery. The in vitro cytotoxicity experiment showed that magnetic nanoparticles did not have the cytotoxic effect and were biologically compatible, to wit that there is potential for biomedical application. In addition, it was found that the IC50 of the nanocapsulated cisplatin in Fe_3O_4 modified with PCL-PEG-PCL copolymer on A_{549} cancerous cell line depends on time.

In vitro cytotoxicity

cytotoxicity assay (MTT assay) data analysis displayed that IC50 of Cisplatin-PCL-PEG- Fe_3O_4 on the A_{549} cancerous cell line is 15 g/ml, 8 g/ml, 2.5 g/ml respectively for 24, 48 and 72 (Table 1)

Following diagrams indicates that IC50 of Fe_3O_4-PCL-PEG-Cisplatin on A_{549} lung cancer cell line depends on time and dose. Additionally, as is shown in Fig. 3, IC50 for pure Cisplatin are 50 g/ml, 22 g/ml, and 8 g/ml respectively for 24, 48 and 72h exposure times (Fig. 6). Therefore, there is a need for further study in the future of this new formulation of cisplatin.

Table 1: The values of IC50 obtained for one Cisplatin-PCL-PEG- Fe_3O_4 and pure Cisplatin

Incubation time (h)	IC50 values (nM) Mean ± SD	
	Free Cisplatin	Cisplatin loaded PCL-PEG
24	50	15
48	22	8
72	8	2.5

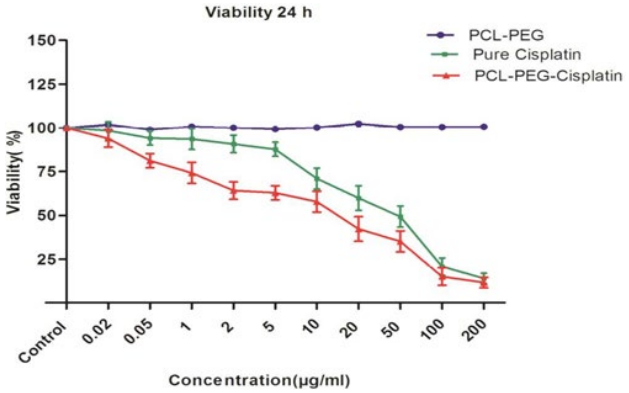

Fig. 6.a: The results of treatment with dilutions of block copolymers, pure and copolymer containing cisplatin in 24 hours

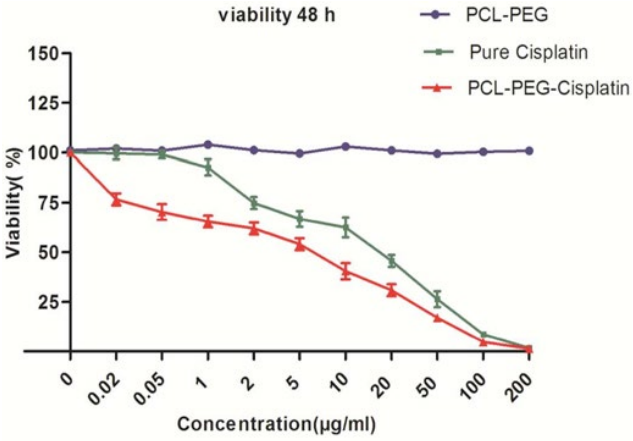

Fig. 6.b: The results of treatment with dilutions of block copolymers, pure and copolymer containing cisplatin in 48 hours.

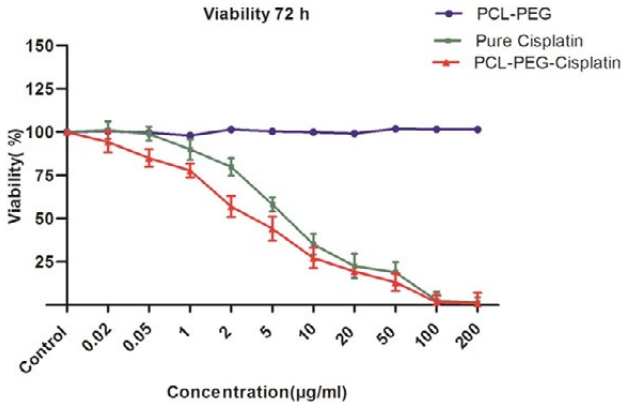

Fig. 6.c: The results of treatment with dilutions of block copolymers, pure and copolymer containing cisplatin in 72 hours.

Characterization of synthesized Nanoparticles

To examine the crystal structure of the magnetic nanoparticles Powder X-ray diffraction (Rigaku D/MAX-2400 X-ray diffractometer with Ni-filtered Cu Ka radiation) was utilized. The size and shape of the nanoparticles were determined using SEM. The sample was solved in ethanol and a small drop was spread onto a 400 mesh copper grid. The infrared spectra were registered using FTIR spectrophotometer (Perkin Elmer series, Waltham, MA), and the sample and KBr were pressed to form a tablet.

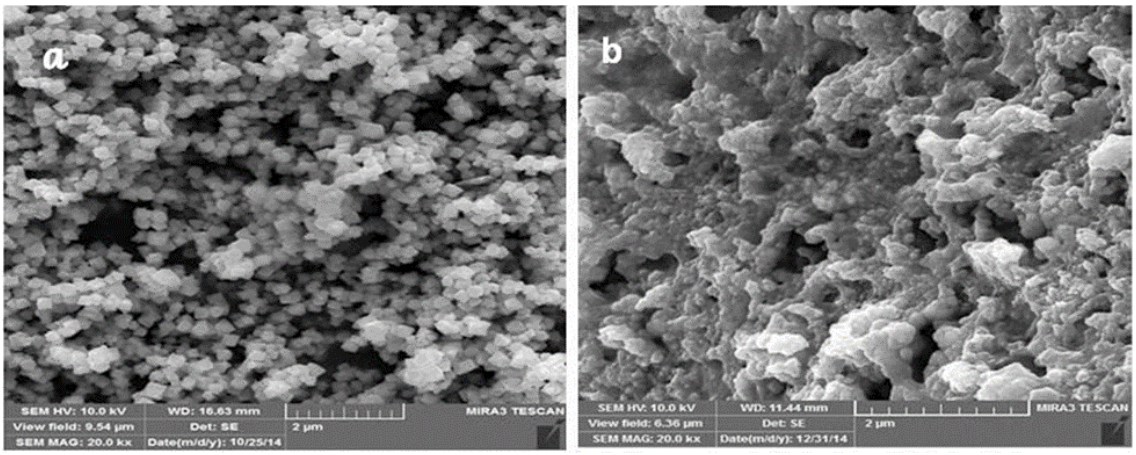

Fig. 7: SEM images of magnetic nanoparticles (40.0 kx) (a) SEM images of Cisplatin-encapsulated magnetic nanoparticles modified with PCL-PEG copolymer (b).

Diffraction patterns of X-ray

The crystalline structure of the synthesized magnetic nanoparticles was analyzed by XRD. The value of θ_2 was considered in the range of 20 to 100. As expected, the diffraction pattern shows a cubic spinel structure for magnetite. Five peaks respectively 220, 311,400, 422, 511, 440, were determined which are consistent with the spectral lines presented in the references. In Fe_3O_4 nanoparticle crystals, the most severe reflection peak is 311, which determines the average size of crystals, using the 'Debye–Scherrer equation ($D_{hkl} = 0.9\lambda$ (β Cos θ)) nanoparticle size was found to be about 9 nm. In this formula, β represents half the width of the XRD diffraction and λ is equal to 0.154 nm and θ is equal to half the diffraction angle of θ_2.

Nanoparticles Size and Size distribution (SEM)

The surface morphology of the nanoparticles was recorded using SEM. The graph of pure nanoparticles (Fig. 7.a) and Cisplatin-encapsulated magnetic nanoparticles modified with PCL-PEG copolymer (Fig. 7.b) are indicated. The photograph shows that the nanoparticles were well accumulated that was because the nano size is about 10nm. After encapsulating and modifying magnetic nanoparticles, the size of the particles changed to 50-70 nm and scatter of the particles was significantly enhanced that using electrostatic repulsion force and a steric barrier between the copolymer chains on the encapsulated nanoparticles is justifiable.

CONCLUSIONS

To reduce the mortality rate of lung cancer, the spread of new therapies, early diagnosis and even prevention of this cancer is of particular importance. Common cancer treatments such as radiation therapy and laser therapy, chemotherapy, surgery, etc. have not had a significant change in the prevention of metastases or the treatment of the disease. Chemotherapy, which is the main body of the treatment, uses strong anti-cancer medicines that are transmitted through the bloodstream (20). Eating or injecting these medicines into the bloodstream will disrupt the ability to make new DNA in cancer cells or prevent the complete division of cancer cells. Since these agents cause inhibition of DNA transcription, they also cause many complications in the body's proliferating cells, such as Hair follicle (hair loss), gonads (infertility), intestines (diarrhea and colitis), bone marrow (cytopenia) etc. There is also the risk of developing cancer from the side effects of these medicines. The use of systems and nanocarriers has been considered for reducing the medicine resistance and side effects of chemotherapy medicines because of the significant difference in the IC_{50} of these systems(42). Clinical applications of cisplatin encounter with challenges such as a very low solubility in biological environments, extensive side effects and a completely non-selective performance in the face of normal and cancerous cells. Which can be used to overcome this limitation in the form of nanocapsules with biocompatible polymers such as PCL, PEG, PLGA, etc. The combination of cisplatin with Poly Lactic-co-Glycolic Acid (PLGA) polymerase in A_{549} cell line has been investigated and has shown an increase in the toxicity and anti-cancer effects of the combination and a reduction in the IC_{50} of the

nanocapsulated medicine compared with the pure medicine(43, 44). Our goal in this study was to eliminate the limitations of cisplatin with the use of the intelligent nano-medicine delivery system. In order to better comparison and observation on the effect of nanoparticles on medicine delivery and anti-cancer effects, the A_{549} cell line was treated with nanocapsulated cisplatin these and pure cisplatin. The MTT assay test showed that the Fe_3O_4 -PCI-PEG copolymer has no toxic and fatal effects on the cancer cell line and is completely biocompatible. As well as, the comparison of results clearly showed that the toxicity and degradation of nanocapsulated cisplatin compared to pure cisplatin had a remarkable effect and IC_{50} was significantly reduced at 24, 48 and 72 hours. That way, IC50 for pure cisplatin at 24, 48 and 72 hours, 50, 22 and 8 µg/ml, and for nano-capsulated cisplatin Respectively 15, 8 µg / ml and 2.5 µg/ml was obtained. The results of the study showed that nanocapsulated cisplatin had a significant cytotoxic and anticancer effect on the A_{549} cell line in-vitro, and it can be concluded from this and previous research(44) that the magnetic copolymer of poly-caprolactone-polyethylene glycol has a forceful toxic effect on lung cancer cells, and this complex can play an important role in treating this cancer. The study showed that Fe_3O_4 -PCL-PEG nanoparticles can be used as a suitable carrier to the preparation of cisplatin nano-formulation for targeted cancer treatment since there is a good balance between the size and incidence of encapsulation. The loading of cisplatin in Fe_3O_4 -PCL-PEG nanoparticles improves medicine delivery and it can be concluded from the results that cisplatin in pure and nanocapsulated mode produces cytotoxic effects on the lung cancer cell line, but in nanocapsulated form under similar conditions at low concentrations It has more potent cytotoxic effects, and these effects are dose-related and time-dependent. The results of this study were in line with previous research also the positive effects of nanoparticles on targeted medicine delivery showed that nanoparticles loaded medicine can improve solubility, gradual and continuous release of the medicine, increased medicine delivery and improved anti-cancer effects of the combination.

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CONFLICT OF INTEREST

The authors reported no declaration of interest. The authors alone are responsible for the content and writing of the paper.

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