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The comparison between effects of free curcumin and curcumin loaded PLGA-PEG on telomerase and TRF1 expressions in calu-6 lung cancer cell line

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Abstract

lung cancer is the most common cancer in men still now. Telomerase is responsible for cancerous cells immortality and is a suitable target for cancer therapy. TRF1 is a modulator for telomerase activity. It is necessary to find more efficient and safer anticancer drugs. Curcumin is a natural polyphenol which has many anticancer effects but it has hydrophobic structure and low solubility in water. PLGA-PEG nanoparticles was used to comprise effects of free curcumin and curcumin loaded PLGA-PEG on telomerase and TRF1 expressions in lung cancer cell line. ¹H NMR, FT-IR and SEM confirmed PLGA-PEG structure and curcumin loading on it. Then, cytotoxic effects of free curcumin and curcumin loaded PLGA-PEG determined by MTT assay. mRNA expression levels of hTERT and TRF1 was determined by Real-time PCR. MTT assay data analysis indicated that curcumin cytotoxicity is dose and time-dependent. Curcumin loaded nanoparticles showed IC₅₀ values in lower concentration in comparison to free curcumin. Curcumin loaded PLGA-PEG decreased hTERT expression and increased TRF1 expression more than pure curcumin. Our study demonstrates curcumin loaded PLGA-PEG promises a natural and efficient system for anticancer drug delivery to fight lung cancer.

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Introduction

In 2008, lung cancer accounts for the most common cancer deaths in males and is the second cause of cancer deaths in females (Cagle & Allen, 2012). Lung cancer is the cause of 13 % (1.6 million) of all cancer cases and 18% (1.4 million) of all deaths in 2008 (Jemal *et al.*, 2008). The World Health Organization has predicted that lung cancer mortality will increase in industrial countries, mainly because of smoking and unhealthy diet (Świątkowska, 2007). Lung cancer is categorized in two types based on aggressive biology and early metastasis; Small cell lung cancer (SCLC) and Non-small cell lung cancer (NSCLC) (Noguchi *et al.*, 1995). Various risk factors affect the lung cancer, including smoking, alcohol consumption, high-fat diet, physical inactivity and excess body weight (Świątkowska, 2007).

Many studies have shown that telomerase activity cause immortalization and unlimited proliferation of cancer cells. Telomerase replicate telomeric DNA sequences and makes cells immortal. Telomerase activity is seen in more than 90 % of cancer cells. Thus, inhibition of telomerase in immortal cancer cells resulting in cell death by apoptosis because of telomeres shortening (Cong, Wright, & Shay, 2002). Telomerase has two major and several minor subunits that one of the main subunits called hTERT (human Telomerase Reverse Transcriptase) which is a catalytic subunit and another is called hTERC (human Telomerase RNA Component) which is an RNA template (Masutomi *et al.*, 2003). hTERT is a reverse transcriptase and hTERC plays a role in telomeres replication as a template (Y. Liu, Dong, Tian, & Liu, 2012) which expresses in all normal and cancerous cells but after fetal period hTERT expresses only in stem cells or cancer cells (Cong *et al.*, 2002). TRF1 is a telomere binding protein and inhibits telomerase which its gene's upregulation results in telomere shortening and downregulation causes more telomere replication and cell immortality (Broccoli *et al.*, 1997).

Three major strategies in cancer treatment are surgery, radiotherapy and chemotherapy (Brannon-

Peppas & Blanchette, 2004). Natural chemicals derived from plants are more effective in cancer chemotherapy and have less side effects on human body. Plants secondary metabolites are useful sources for novel and efficient anti-cancer agents. These small molecules have more stability and are free of chemical and biological contaminants (Cotugno *et al.*, 2012). Polyphenols are a group of these natural metabolites and have antioxidant properties (Namratha *et al.*, 2013). Curcumin is a polyphenol and major component of *Curcuma longa*, known as turmeric (Wilken, Veena, Wang, & Srivatsan, 2011) and has many useful effects against cancer including chemoprevention, apoptosis activation, anti-angiogenesis, anti-proliferation and metastasis inhibition (J. Liu *et al.*, 2013).

Recent advances in medical nanotechnology have resulted in new developments in cancer drug delivery. Drugs encapsulation in nanoparticles with specific characteristics have improved drug target delivery in to cancer cells. Polylactide-co-glycolide (PLGA) is a polymeric nanoparticle approved by FDA because of its biocompatibility and degradation into natural metabolites as well as its safety for human use. These nanoparticles must be uptaken by the cancer cells and many factors influence their internalization. One factor is surface charge, so that the particles with low or no surface charge are easily captured by the reticulo-endothelial system (RES), mainly in the liver and spleen. Increasing the hydrophilicity on the nanoparticles surface is a solution to overcome this problem. Adding a hydrophilic molecule on the surface can prevent nanoparticles of being trapped by the RES. Recently, it is reported that PLGA nanoparticles uptaken by the RES are dramatically reduced by surface modification with polyethyleneglycol (PEG) and these nanoparticles have more half-life in the circulation. Several investigations demonstrated no cytotoxicity for PEGylated PLGA particles and cell viability was at least 94 % (Pamujula *et al.*, 2012; Fernández-Carballido *et al.*, 2008).

Despite different therapeutic strategies, lung cancer is

still one of the most common cancers in the world, so more investigations are needed to find an efficient and safe approach for improvement in current chemotherapies to counteract this malignancy. The objective of present study was to investigate the efficiency of pure curcumin and curcumin loaded PLGA-PEG on telomerase and TRF1 expressions in lung cancer cell line.

Materials and methods

Materials

D, L-lactide and glycolide, PEG (6000), stannous octoate (Sn (Oct)₂: stannous 2-ethylhexanoate), polyvinyl alcohol (PVA), dichloromethane (DCM) were from Sigma-Aldrich (USA). Scanning electron microscopy (SEM) measurements were performed using KYKY model EM3200. An ultraviolet-visible 2550 spectrometer (Shimadzu, Tokyo, Japan) was applied to determine the drug-loading capacity and release behavior. A Perkin Elmer series FTIR (Fourier Transform Infrared) was used to record infrared spectra in real-time. Using a Bruker DRX 300 spectrometer, ¹H NMR (Hydrogen-1 nuclear magnetic resonance) spectra was recorded in real-time operating at 300.13 MHz. A homogenizer (Silent Crusher M, Heidolph Instruments GmbH, Schwabach, Germany). Using a rotary (Rotary Evaporator, Heidolph Instruments, Hei-VAP series) organic phase was vaporized.

Calu-6 lung cancer cell line (code: C431) was obtained from Pasteur Institute of Iran. Primers was purchased from Takapouzist. Curcumin powder was from Merck (Germany). Fetal bovine serum (FBS), trypsin-EDTA and RPMI-1640 were from Gibco, Invitrogen (UK). 3(4, 5-dimethylthiazol-2-yl) 2, 5-diphenyl-tetrazolium bromide (MTT) streptomycin and penicillin G and dimethyl sulphoxide (DMSO) were all obtained from Sigma-Aldrich (USA). RNX-plus kit purchased from CinnaGen (Iran) and was used to extract total RNA. Nanodrop spectrophotometer was Bio Photometer. First Strand cDNA Synthesis kit was obtained from Thermo Scientific. Real-time PCR master mix was from Takara. Real-time PCR was done using Corbett (Rotor Gene 6000).

Synthesis of PLGA-PEG triblock copolymer

Using a melt polymerization procedure under vacuum, PLGA-PEG copolymers with PEG₆₀₀₀ was prepared and stannous octoate [Sn (Oct)₂: stannous 2-ethylhexanoate] was applied as a catalyst. DL-lactide (1.441 g), glycolide (0.285 g) and PEG₆₀₀₀ 0.77 g (45% w/w) were heated in a bottleneck flask to 140 ° C under a nitrogen atmosphere until melting was completed. The molar proportion of DL-lactide and glycolide was 3:1. Then 0.05% (w/w) stannous octoate was added and the reaction mixture temperature was increased to 180 ° C. The temperature was maintained for 3 hours. Using vacuum, polymerization was carried out. The copolymer was dissolved by dichloromethane and precipitated in ice-cold diethyl ether. A triblock copolymer of PLGA-PEG was synthesized using ring opening polymerization of DL-lactide and glycolide in presence of PEG₆₀₀₀.

Using a Bruker AM 300.13 MHz spectrometer, the ¹H NMR spectra were recorded in CDCl₃. The FTIR spectrum was attained from a neat film cast of the chloroform copolymer solution between KBr tablets. A Waters Associates (Milford, MA) Model ALC/gel permeation chromatography 244 apparatus was applied to gel permeation chromatography in dichloromethane performance.

SEM was used to determine size and shape of PLGA-PEG nanoparticles.

Curcumin loading and determination of its encapsulation efficiency 20 mg of curcumin was dissolved in 3 mL methanol. In short, 120 mg of nanoparticles were dispersed in 10 mL dichloromethane solution. Polyvinyl alcohol (PVA) was added to this solution and whole solution was stirred for 2 minutes. Then dichloromethane was evaporated using a rotary evaporator and the remained solution was centrifuged in 9000 rpm for 25 minutes. The supernatant was isolated and used for compare with the total amount of curcumin to determine curcumin encapsulation efficiency of the nanoparticles. The amount of unencapsulated

curcumin in supernatant was measured using an ultraviolet 2550 spectrophotometer (Shimadzu) with an absorbance peak in 420nm. This method allow analysis of a curcumin solution with exclusion of most interfering substances. The percent of curcumin encapsulated on the nanoparticles was measured by the difference between the total amount (OD₁) used to prepare the nanoparticles and the amount of curcumin remained in the supernatant (OD₂), using the following formula:

$$\text{Encapsulation efficiency \%} = [(OD_1 - OD_2) / (OD_1)] \times 100\%$$

In vitro drug release kinetics study

3 mg of curcumin loaded nanoparticles were dispersed in 3 mL of phosphate-buffered solution (pH 7.4) and acetate buffer (pH 5.8, the pH value for investigate pH-dependent and pH sensitivity of compound release kinetics) to study the drug release profile of the prepared curcumin loaded PLGA-PEG nanoparticles. Samples were incubated at various temperatures (37°C & 40°C). At selected time intervals, 3 mL sample was removed and same volume was replaced by adding 3 mL of new phosphate-buffered solution and acetate buffer to each sample. After the experiment, ultraviolet spectrophotometry was applied to determine the amount of curcumin release.

Cell culture

For this experimental study, calu-6 lung cancer cell line (Pasteur institute, Iran) were cultured in RPMI-1640 medium (Gibco, Invitrogen, UK) supplemented with 10-20% heat inactivated fetal bovine serum (FBS) (Gibco, Invitrogen, UK), 2 mM L-glutamine, Penicillin G (80 mg/mL) (Merck Co, Germany), Streptomycin (50 mg/mL) (Merck Co, Germany) and NaHCO₃ (2 g/mL), at 37°C and in 5% CO₂.

Cell viability and MTT-based cytotoxicity test

Cells in the exponential phase of growth were exposed to free curcumin and curcumin loaded PLGA-PEG. Cytotoxic effect of free curcumin and curcumin loaded PLGA-PEG was studied by MTT assay in 24, 48 and 72 h after treatment. 10000 cells/well were

cultivated in a 96-well plate (Coastar from Corning, NY) and after 24 h incubation were treated with different concentrations (2.5-70µM) of free curcumin and curcumin loaded PLGA-PEG for 24, 48 and 72 h in the quadruplicate manner. After these different exposure durations, medium was removed and then the cells were fed with 200 µL of fresh medium. Cells were incubated for 24 h, then 50µL of 2 mg/ml MTT (Sigma co, Germany) dissolved in PBS was added to each well and plates were covered with aluminum foil and incubated for 4 h. Next, wells content was removed and 200 µL pure DMSO and 25µL Sorensen's glycine buffer were added. Finally, the absorbance measurement was determined at 570 nm using an ELISA plate reader (with a reference wavelength of 630 nm).

Total RNA extraction, cDNA synthesis and Real-time PCR

After 72 treatment with different concentration of pure curcumin and curcumin loaded PLGA-PEG, total RNAs was extracted. Thus, nanodrop was used to determine their purity and concentration. Electrophoresis on a 1% agarose gel confirmed total RNAs integrity. RNA samples was changed to complementary DNA (cDNA) using First strand cDNA synthesis kit. For each reaction sample 1 µg/µL total RNA was used. Each reaction sample was prepared and contains 1 µL of oligo dT primer, 10 µL of nuclease-free water, 4 µL of 5X Reaction Buffer, 1 µL RiboLock RNase inhibitor, 2 µL of dNTP Mix and 1 µL of RevertAid M-MuLV Reverse Transcriptase. These reaction mixtures was incubated under following conditions: 37°C, 15 minutes (Reverse Transcription); 85°C, 5 sec (inactivation of reverse transcriptase with heat treatment); 4°C. Real-time PCR was used to determine hTERT and TRF1 expression levels. For Real-time PCR reaction, 2 µL (4 picomolar) of forward and reverse primers of hTERT (5'-GTCTGGAGCAAGTTGCAAAG-3', 5'-TGACCTCTGCTTCCGACA-3'), TRF1 (5'-TCTCTCTTTGCCGAGCTT-3', 5'-ACTGGCAAGCTGTTAGACTGG-3'), β-actin (5'-TCCCTGGAGAAGAGCTACG-3', 5'-GTAGTTTCGTGGATGCCACA-3'), 6.5 µL of Real-

Time PCR master mix, 2 μL of deionized water and 2 μL of cDNA sample was used. For hTERT, TRF1 and β -actin, amplicons sizes was 168 bp, 161 bp and 181 bp, respectively. Equal amounts of each RNA sample were prepared, in parallel with each other, hTERT, TRF1 and β -actin were amplified using Real-Time PCR in triplicate. The reaction mixture was incubated under the following conditions: 95°C, 5 minutes, 1 cycle (Holding step); 95°C, 15 seconds, 40 cycles (Denaturation); 60°C, 15 seconds, 40 cycles (Annealing); 72°C, 30 seconds, 40 cycles (Extension); 72-95°C, 1 cycle (Melting).

Statistical analysis

Statistical analysis was done using SPSS 16. Differences between control and treated samples was calculated using t-test method and $p < 0.05$.

Results

Table 1. IC₅₀ values of curcumin and curcumin loaded PLGA-PEG at different times of incubation.

| IC ₅₀ values (μM) | | |
|---|------------------|--------------------------|
| Mean \pm SD | | |
| Incubation | Free curcumin | Curcumin loaded PLGA-PEG |
| 24h | 27.32 \pm 1.03 | 14.47 \pm 1.25 |
| 48h | 11.36 \pm 1.8 | 6.78 \pm 1.65 |
| 72h | 8.13 \pm 1.56 | 5.22 \pm 1.36 |

Table 2. Real-time PCR data analysis for hTERT expression.

| number | sample | C _T | IC.C _T | ΔC_T | $\Delta\Delta\text{C}_T$ | Relative expression |
|--------|--|----------------|-------------------|--------------------|--------------------------|---------------------|
| 1 | Control | 22.12 | 12.31 | 9.81 | 0 | 1 |
| 2 | Control PLGA-PEG | 22.16 | 11.36 | 10.8 | 0.99 | 0.5 |
| 3 | Pure curcumin 8.13 μM | 22.79 | 12.7 | 10.09 | 0.28 | 0.82 |
| 4 | Pure curcumin 11.36 μM | 24.07 | 13.03 | 11.04 | 1.23 | 0.42 |
| 5 | Pure curcumin 27.32 μM | 23.95 | 12.89 | 12.78 | 2.97 | 0.12 |
| 6 | Curcumin loaded PLGA-PEG 8.13 μM | 23.95 | 11.96 | 11.99 | 2.18 | 0.22 |
| 7 | Curcumin loaded PLGA-PEG 11.36 μM | 24.33 | 12.02 | 12.31 | 2.5 | 0.17 |
| 8 | Curcumin loaded PLGA-PEG 27.32 μM | 25.7 | 12.37 | 13.33 | 3.52 | 0.08 |

Physicochemical characterization of PLGA-PEG nanoparticles

SEM results showed the surface morphology of nanoparticles. The nanographs for PLGA-PEG copolymers (Fig. 5) and Curcumin loaded PLGA-PEG (Fig. 6) are shown. Using the photograph, it can be observed well aggregation of nanoparticles, which due to size of PLGA-PEG was 55 \pm 6 nm. After

Synthesis and characterization for PLGA-PEG copolymers

¹H NMR spectra that were recorded at RT with a Bruker DRX 300 spectrometer operating at 400 MHz confirmed the basic chemical structure of PEG-PLGA co-polymer. Tetramethylsilane (TMS) as an internal reference was used for measuring Chemical shift (δ) in ppm. Because of the methylene groups of the PEG, there was a large peak at 3.65 ppm. Methyl groups of the D-lactic acid and L-lactic acid repeat units provides overlapping doublets at 1.55 ppm. Attributing to the lactic acid CH and the glycolic acid CH, there were peaks at 5.2 and 4.8 ppm, respectively. High complexity of the 2 peaks was observed, because of D-lactic, L-lactic and glycolic acid sequences difference in the polymer backbone. The structure of copolymer is consistent with the FTIR spectrum. Using FTIR spectroscopy, the structure of PLGA-PEG copolymer was observed.

encapsulation of curcumin on PLGA-PEG nanoparticles, the size of particles change to 300 \pm 6 nm dispersion of the particles was significantly improved.

Drug loading and in vitro releasing

Encapsulation efficiency was 84.5 % and it was determined that 1 mg of PLGA-PEG included 845 μg

of curcumin.

It was found that curcumin releasing is time-dependent and increase in pH 5.8 and temperature 40° C, which are characteristic for cancerous cells environment (Fig. 7).

Cell cytotoxicity (MTT assay)

In this study to evaluate the cytotoxic effect (MTT assay) of free curcumin and curcumin loaded PLGA-PEG, calu-6 lung cancer cell line were treated with different concentration (2.5-50 μ M) of free curcumin and curcumin loaded PLGA-PEG for 24, 48 and 72h.

IC₅₀ after 24 h treatment with free curcumin and curcumin loaded PLGA-PEG was 27.32 \pm 1.03 and 14.47 \pm 1.25 μ M (p < 0.05) respectively (Fig. 8). PLGA-PEG and methanol 1% showed an absorbance value equivalent of 98 and 96% of control respectively. It suggests that PLGA-PEG and methanol 1% have very low effect on the cells. IC₅₀ after 48 h treatment with curcumin and curcumin loaded PLGA-PEG were 11.36 \pm 1.8 and 6.78 \pm 1.65 μ M (p < 0.05) respectively (Fig. 9). After 72 h treatment with curcumin and curcumin loaded PLGA-PEG nanoparticles, IC₅₀ were 8.13 \pm 1.56 and 5.22 \pm 1.36 μ M (p < 0.05) respectively (Fig. 10).

Table 3. Real-time PCR data analysis for TRF1 expression.

| number | sample | C _T | IC.C _T | Δ C _T | $\Delta\Delta$ C _T | Relative expression |
|--------|--|----------------|-------------------|-------------------------|-------------------------------|---------------------|
| 1 | Control | 26.85 | 20.6 | 14.54 | 0 | 1 |
| 2 | Control PLGA-PEG | 25.21 | 11.36 | 13.85 | -0.69 | 1.61 |
| 3 | Pure curcumin 8.13 μ M | 24.43 | 12.7 | 11.73 | -2.81 | 7.01 |
| 4 | Pure curcumin 11.36 μ M | 24.28 | 13.03 | 11.25 | -2.99 | 7.94 |
| 5 | Pure curcumin 27.32 μ M | 23.55 | 12.89 | 10.66 | -3.88 | 14.72 |
| 6 | Curcumin loaded PLGA-PEG 8.13 μ M | 22.94 | 11.96 | 10.98 | -3.56 | 11.79 |
| 7 | Curcumin loaded PLGA-PEG 11.36 μ M | 21.6 | 12.02 | 9.58 | -4.96 | 31.12 |
| 8 | Curcumin loaded PLGA-PEG 27.32 μ M | 20.09 | 12.37 | 7.72 | -6.82 | 112.98 |

Quantitative mRNA analysis

The levels of hTERT and TRF1 genes expressions were measured by Real-Time PCR. Changes in hTERT and TRF1 expressions levels between the control and treated calu-6 cells were normalized to β -actin mRNA levels and then calculated by the 2^{- $\Delta\Delta$ C_T} method. Real-time PCR data analysis indicated that by increasing amount of pure curcumin and curcumin loaded PLGA-PEG, hTERT mRNA level expression would be decreased and TRF1 mRNA level expression would be increased. Curcumin loaded PLGA-PEG decreased hTERT expression and increased TRF1 expression more than pure curcumin (Tables 2&3) and (Figs. 11&12)

Discussion

Controlled and targeted drug delivery is essential for anticancer drugs performance and avoid unintended effects on normal cells. Today, development of new drug delivery systems for anticancer drugs delivery is a major issue in cancer research. Nanoparticles can

cross tissues and organs gaps and uptake by the cells. Controlled release of nanoparticles depends on pH, ions and temperature (Dinarvand, Sepehri, Manoochchri, Rouhani, & Atyabi, 2011).

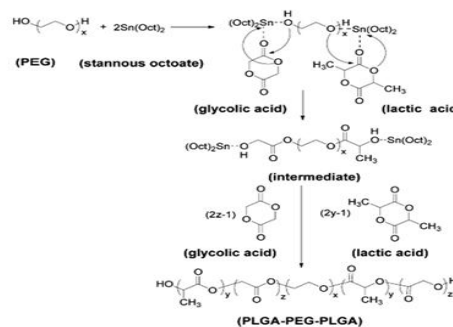


Fig. 1. Mechanism of PLGA-PEG prepared by Sn (Oct)₂ as catalyst.

Curcumin is well-known as an effective and safe anticancer agent (Lin, J. K. and Lin-Shiau, 2001) although its oral administration is limited due to low solubility in water (Sou, Inenaga, Takeoka, & Tsuchida, 2008). Biodegradable nano drug carriers offered new prospects in solving the problem of low

water solubility of hydrophobic drugs (Gou *et al.*, 2011). In this paper, we used PLGA-PEG nanoparticles to increase curcumin solubility in cancer cells aqueous environment.

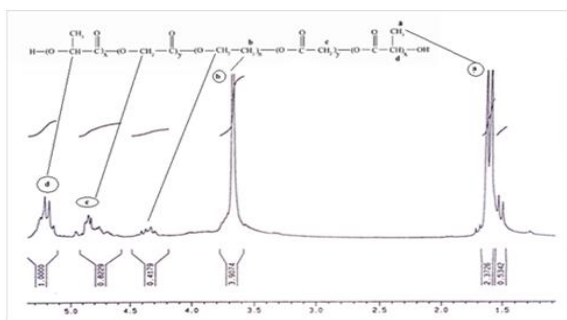


Fig. 2. ^1H NMR spectrum of PEG-PLGA co-polymer.

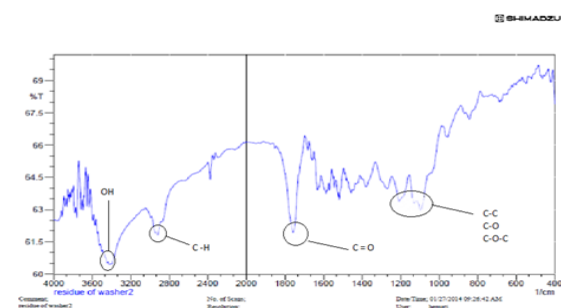


Fig. 3. FT-IR plot of PLGA-PEG.

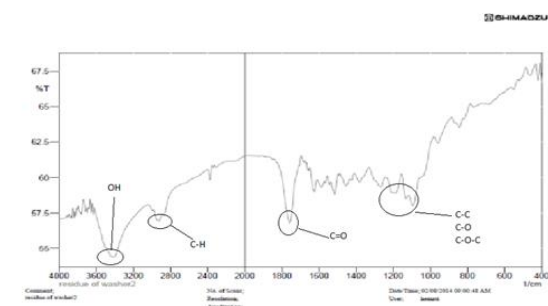


Fig. 4. FT-IR plot of curcumin loaded PLGA-PEG

PLGA-PEG nanoparticles show high potential and possess many applications in new drug delivery systems, already utilized for some drugs delivery (Naksuriya, Okonogi, Schifflers, & Hennink, 2014). In this work, curcumin was encapsulated into PLGA-PEG using double emulsion method (w/o/w). FT-IR, ^1H NMR and SEM analysis confirmed curcumin loading on PLGA-PEG. Curcumin loaded PLGA-PEG nanoparticles had 65 ± 4 nm size and encapsulation efficiency was 84.5 %. Also it showed that curcumin has maximum amount of release in cancer cell-like conditions (40°C , $\text{pH} = 5.8$).

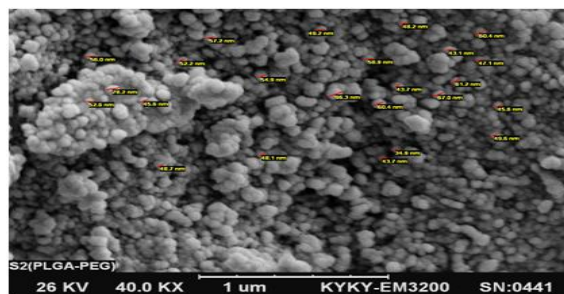


Fig. 5. Scanning electron microscopy of PLGA-PEG.

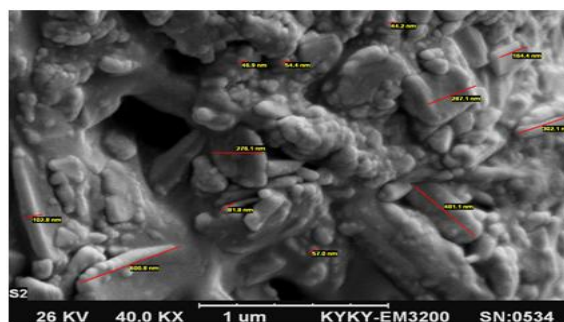


Fig. 6. Scanning electron microscopy of curcumin loaded PLGA-PEG.

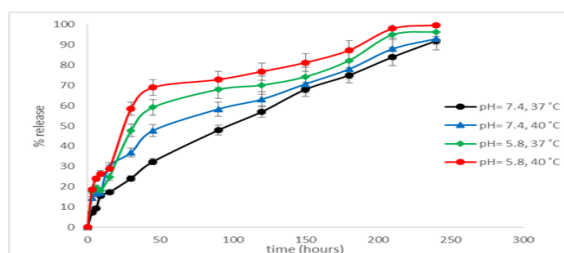


Fig. 7. Curcumin release for different pHs and temperatures.

In previous reports, a number of polymeric nano-carriers were used for curcumin drug delivery. Shaikh *et al* synthesized curcumin loaded PLGA nanoparticles with 264 nm size and 76.9 % encapsulation efficiency. These nanoparticles increased oral bioavailability 9-folds (Shaikh, Ankola, Beniwal, Singh, & Kumar, 2009). Anand *et al* used PLGA-PEG nanoparticles for curcumin delivery. These nanoparticles (80.9 nm) possess 97.5 % encapsulation efficiency. They found curcumin encapsulation into PLGA-PEG enhances cellular uptake, improves performance *in vitro* and better bioavailability *in vivo* (Anand *et al.*, 2010). It was found in recent study, PLGA nanoparticles containing curcumin stop the cell cycle at the G_2/M in the MCF-7 breast cancer cell line (Verderio, Bonetti, Colombo, Pandolfi, & Prosperi, 2013). Moreover, it was

indicated curcumin loaded PLGA nanoparticles cause cellular uptake of this compound by breast cancer cell line (MDA-MB-231) and ovarian cancer cell line (A2780CP) increase 6 and 2-fold, respectively (Yallapu, Gupta, Jaggi, & Chauhan, 2010).

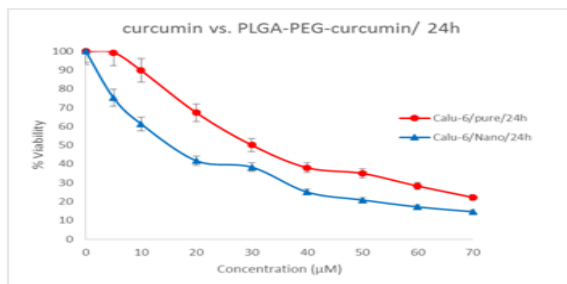


Fig. 8. MTT assay results for 24h. Cytotoxic effect of different concentrations of free curcumin and curcumin loaded PLG-PEG on calu-6 cell line.

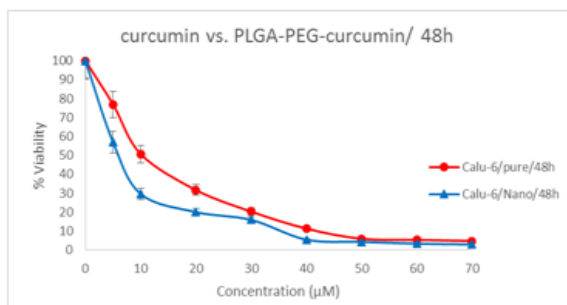


Fig. 9. MTT assay results for 48h. Cytotoxic effect of different concentrations of free curcumin and curcumin loaded PLG-PEG on calu-6 cell line.

Telomerase is a comprehensive and unique target molecule for non-small cell lung cancer (NSCLC) treatment. Although many compounds affects telomerase expression and activity, but many of these compounds has side effects (Mittal, Pate, Wylie, Tollefsbol, & Katiyar, 2004) and developing alternative agents such as natural ingredients is necessary. A study indicated curcumin suppresses telomerase expression in liver cancer (Bel7402), leukemia (HL 60) and gastric cancer (SGC7901) cell lines (Cui *et al.*, 2006). Our results also confirmed the results of these investigation and showed enhancement of curcumin concentration decreases telomerase expression in lung cancer cell line model. It is confirmed that encapsulation on PLGA-PEG improve drug delivery in cancer cells environment and cause more inhibition of telomerase and more decrease in cancer cell immortality.

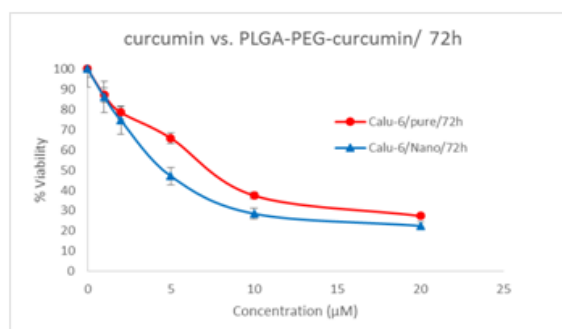


Fig. 10. MTT assay results for 72h. Cytotoxic effect of different concentrations of free curcumin and curcumin loaded PLGA-PEG on calu-6 cell line.

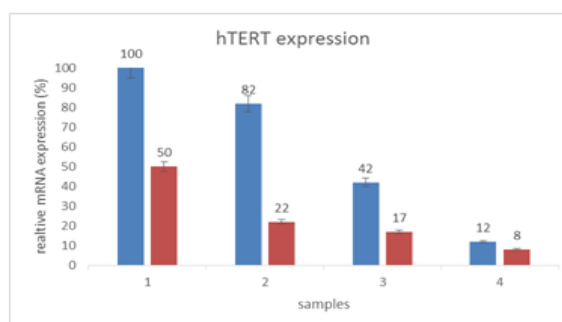


Fig. 11. Expression levels of hTERT. 1. Blue: control/ Red: control PLGA-PEG*2. Blue: pure curcumin (8.13 µM)/Red: curcumin loaded PLGA-PEG (8.13 µM)*3. Blue: pure curcumin (11.36 µM)/Red: curcumin loaded PLGA-PEG (11.36 µM)*4. Blue: pure curcumin (27.32 µM)/Red: curcumin loaded PLGA-PEG (27.32 µM).

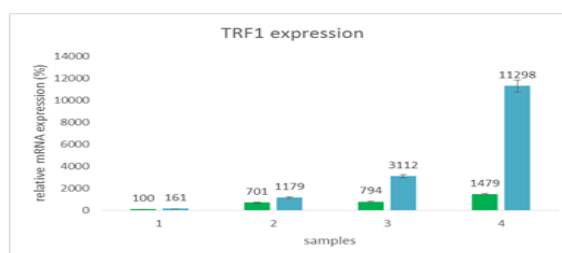


Fig. 12. Expression levels of hTERT. 1. Green: control/ Blue: control PLGA-PEG*2. Green: pure curcumin (8.13 µM)/Blue: curcumin loaded PLGA-PEG (8.13 µM)*3. Green: pure curcumin (11.36 µM)/Blue: curcumin loaded PLGA-PEG (11.36 µM)*4. Green: pure curcumin (27.32 µM)/Blue: curcumin loaded PLGA-PEG (27.32 µM).

TRF1 is a telomerase modulator proteins which any drug effects on its expression was not studied still now and our work is the first study of curcumin effect

on its expression. Our results indicated that curcumin increases TRF1 expression and curcumin encapsulation on PLGA-PEG makes this effect more. The results of this study show that curcumin loaded PLGA-PEG had inhibitory effect on calu-6 lung cancer cell line more than pure curcumin. This inhibition was dose and time dependent. Analysis of our data demonstrate that PLGA-PEG release curcumin dose and time dependently.

To our knowledge, this work is the first application of curcumin loaded PLGA-PEG nanoparticles in calu-6 lung cancer cell line treatment.

Conclusion

Curcumin loaded PLGA-PEG nanoparticles decrease telomerase gene expression and increase TRF1 gene expression and more TRF1 protein synthesis causes telomerase enzyme inhibition more. PLGA-PEG containing curcumin can be used for developing new and effective drug delivery systems to fight lung cancer.

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Declaration of interest

The authors report no declarations of interest.

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