**Research Paper**

# Flavonoid Delivery to the Target Cells through the Synthetic Nanoparticles

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**Abstract**

In this research, poly (lactide-co-glycolide)-block-poly (ethylene glycol), (PLGA-PEG) nanoparticles (NPs) of less than 195 nm in diameter containing of Naringenin (NRG) a naturally flavonoid were synthesized. Encapsulated form NRG improves its medical properties and solubility. The therapeutic efficacy of the encapsulated naringenin (NRG-NPs) and NRG on human lung epithelial (A549) and mouse mammary (4T1) carcinoma cells proliferation was determined by MTT assays. The cytotoxicity potency was rated as follows: NRG-NPs > NRG. The antioxidant effects of the NRG and NRG-NPs were also determined by FRAP method. Our results show that NRG-NPs are cytotoxic compounds for cancer cells and anti-cancer effect can be attributed to the presence of Fe chelatory and antioxidant effects of NRG-NPs.

## 1. Introduction

Naringenin (NRG, 4', 5, 7- trihydroxy flavanone, (Fig 1a), a naturally occurring flavonoid, and aglycone of naringin, is widely present in citrus fruits, tomatoes, cherries, grapefruit and cocoa [1]. It is well known for various biological actions, such as antioxidant, anti- inflammatory and anti-carcinogenic effects. In the best-case scenario, only 15% of ingested naringenin will get absorbed in the human gastrointestinal tract. Yet it suffers from biopharmaceutical restrictions due to its poor water solubility which results in poor absorption and short half-life about 2 hours [2,3]. Encapsulated form flavonoid improves its medical properties and solubility. Previous studies showed that dendrosome, a diblock nanostructure made by oleic acid (OA) and polyethylene glycol (PEG, 400 Dalton) with anticancer and proapoptosis effects, is a suitable option for curcumin encapsulation [4, 5].

Over the past few decades, biodegradable polyesters, such as poly (lactic acid) (PLA), poly (glycolic acid) (PGA), and poly (lactic-co-glycolic acid) (PLGA), have been extensively studied for a wide variety of pharmaceutical and biomedical applications [6]. The biodegradable polyester family has been regarded as one of the few synthetic biodegradable polymers with controllable biodegradability, excellent biocompatibility, and high safety [7]. Extensive studies throughout the world have produced encouraging results demonstrating many desirable, unique properties of PLGA-PEG block copolymers (Fig 1b).

## 2. Materials and Methods

### 2.1. Materials

PLGA-PGE (Resomer 50105) was obtained from Behringer Intgeihim (Germany). meso Naringenin

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(99% purity), sodium cholate, Butylated hydroxytoluene (BHT), MTT (3-[4,5-dimethylthiazol-2-yl] 2, 5diphenyltetrazolium bromide) and other materials were obtained from Sigma-Aldrich (GmbH, Sternheim, Germany). The human lung epithelial (A549) and mouse mammary (4T1) carcinoma cell lines were procured from Pasteur Institute of Iran (Pasteur Institute, Tehran, Iran).
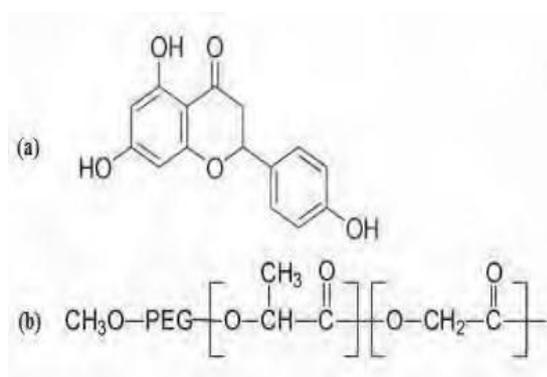


**Figure1.** The chemical structure of (a): Naringenin and (b): PLGA-PEG

## 2.2 Methods

### 2.2.1 NRG-NPs Preparation
PLGA– PEG nanoparticles loaded with NRG were prepared using the double emulsion method [8], with minor modifications. An aqueous solution of NRG (5ml, 2.5 mg / ml) was emulsified in 2 ml dichloromethane, in which 100 mg of the copolymer had been dissolved, using probe sonication Scientific; model (Hielscher, UP 4000 S, Germany) at 10 W for 45 s. This w / o emulsion was transferred to an aqueous solution sodium cholate (6 ml, 12mM) and the mixture was probe sonicated at 18 W for 1 min. The w/o/w emulsion formed was gently stirred at room temperature until the evaporation of the organic phase was complete. The nanoparticles were purified by applying two cycles of centrifugation (28 000 rpm for1h in a Microelite RF, USA) and reconstitution with deionized and distilled water. Finally the nanoparticles were filtered using 0.22 μm syringe filter to remove non encapsulated NRG. The complex was then lyophilized and drug loading and encapsulation efficiency of NRG-NPs micelles were quantified.

### 2. 2. 2. The Total Antioxidant Capacity (TAC) Assay
The total antioxidant capacity (TAC) of the NRG and NRG-NPs were determined by FRAP (Ferric Reducing Antioxidant Power) method, a simple, speedy and repeatable method, which can be used to the assay of antioxidants in plasma or botanicals [9]. The FRAP assay based on the ability of antioxidant compounds to reduce complex [Fe(III)–

TPTZ)] to [Fe(II)–TPTZ] which gives a blue color with an absorbance maximum at 593 nm. The FRAP reagent composed of 10 mM TPTZ solution in 40 mM HCl, 20 mM $FeCl_3$ solution and 300 mM acetate buffer (pH= 3.6) in a ratio of 1:1:10 (v/v). 50 μl of NRG-NPs and NRG (100 μg/ml) were added to 3 ml of freshly prepared FRAP reagent and reaction mixtures incubated at 37 °C for 30 min. Aqueous solutions of ferrous sulfate were used to construct standard curve. Absorbance was determined at 593 nm. Triplicate measurements were taken and the FRAP values were expressed as mmol of Fe (II)/ g dry weight of NRG and NRG-NPs.

### 2. 2. 3. Cell Culture and Cytotoxicity Assay
A549 and 4T1 were grown in Dulbecco's modified Eagle's medium (DMEM; GIBCO, USA) containing FBS (10%, v/v) and antibiotics [penicillin (80 units/mL) and streptomycin (80 μg/mL)] at 37 °C in a $CO_2$ incubator (5% $CO_2$ and 95% relative humidity). In order to evaluate the cytotoxic effect of NRG, NPS and NRG-NPs, Cell viability measured by MTT (3-[4,5-dimethylthiazol-2-yl] 2, 5-diphenyltetrazolium bromide) assay. The MTT assay measures the reduction of a tetrazolium component into an insoluble formazan product by the mitochondria of viable cells. Identical cell numbers ($1 \times 10^5$ cells) in 200 μL DMEM containing 10% FBS were seeded in triplicate on 96-well plates and incubated overnight. Cells were subsequently treated with various concentrations of NRG-NPs, NPs and NRG for 48 h and then 20 μL of MTT (5 mg/mL) was added to each well and incubated for an additional 4 h followed by adding 200 μL of dimethyl sulfoxide (DMSO). The color intensity generated is directly proportional to the number of viable cells. Relative cell viability was then determined using a 96-well plate reader (TECAN, Switzerland) at 540 nm. All experiments were performed in triplicates, standard deviations were calculated. **3.**

## 3. Results and Discussion
Nanoparticles were characterized by scanning Electron Microscopy NRG-NPs had a small size (<195 nm), good encapsulation efficiency (91.26 ± 3.15%), and high drug loading (10.49 ± 0.25%) (Fig. 2 and 3).



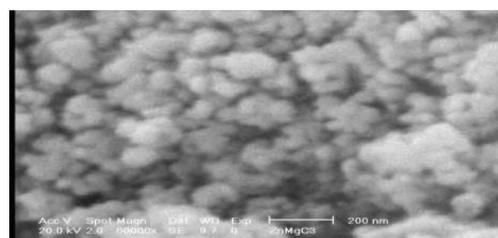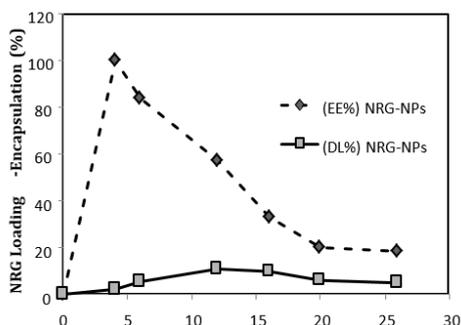**Figure 2.** Scanning electron microscope (SEM) of NRG-NPs

**Figure 3.** Drug loading and Encapsulation efficiency of NRG-NPs

**Table 1.** Total Antioxidant Capacity (TAC)

| Samples | TAC (mmol Fe$^{+2}$/g sample) |
|---|---|
| NRG | 3.87± 0.09* |
| NRG-NPs | 5.11 ± 0.12* |
| *Values expressed are means ± SD | |

Antioxidants protect biological systems from oxidative damage produced by reactive oxygen species (ROS) and are therefore considered as health-promoting compounds in nutrition. The NRG-NPs also showed antioxidant activity with the greater activity of the NRG (Table 1). The antioxidant activity of NRG has been reported by other authors [10,11]. There is a mutual relation between Fe chelatory and antioxidant properties of the compounds. Chelation of iron and quenching of singlet oxygen are the major characteristics of antioxidant activity.

NRG-NPs significantly suppressed the proliferation of A549 and 4T1 cancerous cells in a dose and time dependent manner in comparison with NRG and NPs. Therefore, we showed that IC$_{50}$ value (the concentration of material required to achieve 50% reduction in cell viability in comparison to untreated controls) of the NRG-NPs is significantly lower than NRG. In addition, no significant toxicity was observed for NRG-NPs. NRG-NPs IC50 for 4T1 cells was 25 $\mu$M within 48 h and for A549 cells was 18 $\mu$M (Figure 4). We showed that IC50 of the free NRG is significantly higher than NRG-NPs, and NRG-NPs significantly suppressed cell growth compared to free NRG. In addition, no significant toxicity was observed for NPs. The cytotoxicity potency was rated as follows: NRG-NPs > NRG. There was a correlation between Fe-chelatory and cytotoxicity activities of the NRG-NPs.
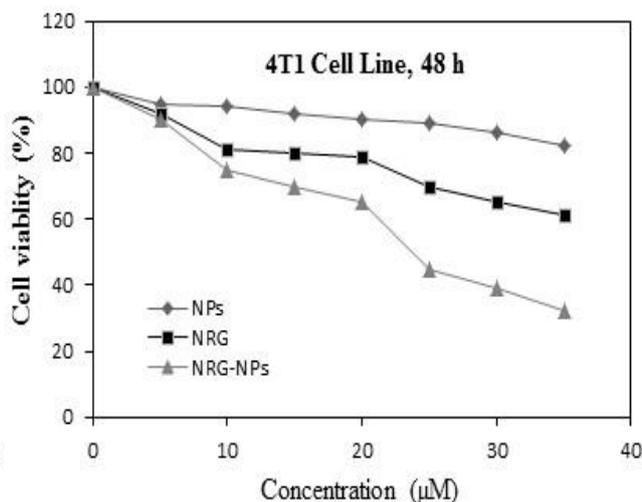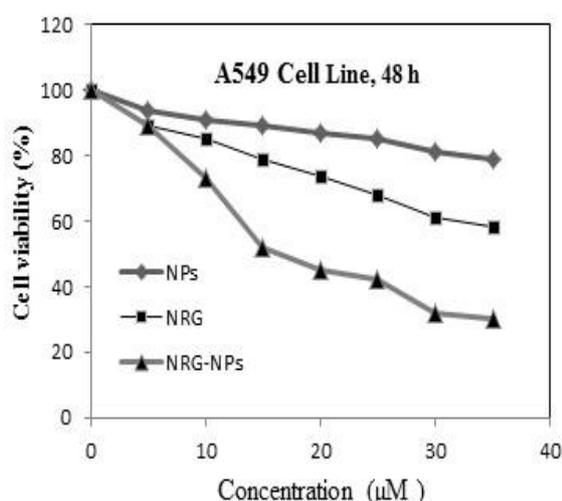


**Figure 4.** Cytotoxic effects of NRG, NPs and NRG-NPs on 4T1and A549 cell lines

In our study, the NRG-NPs significantly suppressed proliferation of human and mouse carcinoma cells in vitro and not only boost NRG solubility and uptake in cell lines but also increase its toxicity on cancer cells. Because of cancer cells rapidly proliferate, these cells have higher requirement for iron than normal cells [12,13]. The increased requirement of cancer cells to iron has led to sensitivity of these cells to cytotoxic effects of Fe. Considering the vital role of iron in cellular proliferation and its potential to mediate deleterious oxidative damage when in excess, Fe-chelating agents provide a promising form of treatment for both iron overload disease and cancer therapy [14]. Due to the problems with the current synthetic drugs, finding natural products with iron-chelating activity could be a good approach for treating cancer.

## 4. Conclusion

Our results show that NRG-NPs are cytotoxic compounds for A549 and 4T1 cancer cell lines and anticancer effect can be attributed to the presence

of Fe chelatory and antioxidant effects of NRG-NPs. These nanocarriers are effective in suppressing tumor growth in vitro and reducing anticancer drug side effects.

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