Enhanced Lycopene Delivery Using Estrogen Receptor Targeted Nanoparticles For Breast Cancer Therapy

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Abstract: ***Lycopene has extensively been shown to inhibit tumor growth in nude mice bearing Breast. However, high tumor-suppressive Lycopene dosages encumber the development of oral controlled-release formulations because of a short biological half-life (< 2 h), poor absorption, low aqueous solubility, and extensive first-pass metabolism. Here, we present the design, fabrication, optimization, characterization, and biologicalevaluation of estrone-conjugated lycopene -loaded gelatin nanoparticles for targeting estrogen receptor- positive breast cancer MCF-7 cells. Gelatin nanoparticles (GN) were uniformly compact sized, stable***
at physiological pH, and showed good drug entrapment efficiency.

Keywords:Lycopene, nanoparticles,gelatin

1. INTRODUCTION

Chemotherapy for breast cancer uses drugs to target and destroy breast cancer cells. These drugs are usually given directly into a vein through a needle or as a pill. Chemotherapy for breast cancer frequently is used in addition to other treatments, such as surgery, radiation or hormone therapy. Receiving chemotherapy for breast cancer may increase the chance of a cure, decrease the risk of the cancer returning, alleviate symptoms from the cancer or help people with cancer live longer with a better quality of life. If the cancer has recurred or spread, chemotherapy may control the breast cancer to help you live longer. Or it can help ease symptoms the cancer is causing. The biology and behavior of breast cancer affects the treatment plan. Some tumors are smaller but grow quickly, while others are larger and grow slowly[1].Treatment options and recommendations are very personalized and depend on several factors, including:

- The tumor's subtype, including hormone receptor status (ER, PR), HER2 status, and nodal status
- The stage of the tumor
- Genomic markers, such as Oncotype DX™ or MammaPrint™
- The patient's age, general health, menopausal status, and preferences
- The presence of known mutations in inherited breast cancer genes, such as *BRCA1* or *BRCA2*

There may be several benefits to having other treatments before surgery:

- Surgery may be easier to perform because the tumor is smaller.
- Your doctor may find out if certain treatments work well for the cancer.

- You may also be able to try a new treatment through a clinical trial.
- If you have any microscopic distant disease, it will be treated earlier.
- Women who may have needed a mastectomy could have breast-conserving surgery (lumpectomy) if the tumor shrinks enough before surgery.

After surgery, the next step in managing early-stage breast cancer is to lower the risk of recurrence and to get rid of any remaining cancer cells in the body. These cancer cells are undetectable with current tests but are believed to be responsible for a cancer recurrence as they can grow over time. Treatment given after surgery is called "adjuvant therapy." Adjuvant therapies may include radiation therapy, chemotherapy, targeted therapy, and/or hormonal therapy (see below for more information on each of these treatments).

Whether adjuvant therapy is needed depends on the chance that any cancer cells remain in the breast or the body and the chance that a specific treatment will work to treat the cancer. Although adjuvant therapy lowers the risk of recurrence, it does not completely get rid of the risk. Molecularly targeted therapies have recently emerged as an attractive approach to overcome the lack of specificity of conventional chemotherapeutic agents[2,3].

Lycopene is a natural pigment synthesized by plants and microorganisms but not by animals. Lycopene is the red colored pigment abundantly found in red colored fruits and vegetables such as tomato, papaya, pink grapefruit, pink guava and watermelon. This red colored pigment was first discovered in the tomato by Millardet in 1876. It was later named lycopene by Schunck . Because of its non-polarity, lycopene is lipophilic, insoluble in water, and can be dissolved only in organic solvents and oils[4].

It is a carotenoid, an acyclic isomer of b-carotene, and has no vitamin A activity. It is a highly unsaturated, straight chain hydrocarbon containing 11 conjugated and two non-conjugated double bonds. This unique nature of the lycopene molecule makes it a very potent antioxidant[5]. Estrogen receptors (ERs) are selectively overexpressed up to B80% in human breast cancers compared with normal breast epithelia [6]. ERs are a group of proteins that are activated by the hormone, estrogen. ER is a member of the nuclear hormone family of intracellular receptors, whereas estrogen G protein-coupled receptor GPR30 is a G protein-coupled receptor. ERs are generally desiconjugated gelatin nanoparticlesated as cytoplasmic receptors in their unliganded state, but a fraction of the ERs also resides in the nucleus. Once activated by estrogen, the ER is able to translocate into the nucleus and subsequent binding with DNA regulates the activity of different genes [7]. Estrone (ES) binds preferentially to the ER-a receptor found in breast epithelial cells, whereas other ligands such as estriol, raloxifene, and genistein bind to the ER-b receptor. It has been reported that ES sulfate promotes the growth of MCF-7 cells by converting into estradiol by catalytic activity of ES sulfatase and 17b-hydroxysteroid dehydrogenase [8]. MCF-7 cells convert the physiological concentration of ES sulfate into free estradiol, which further stimulates cell growth [9]. However, other analogues of ES such as 2-ethylestrone and 2-ethylestrone-3-O-sulfamate induce mitotic arrest and apoptosis in MCF-7 and CAL-51 breast cancer cells, respectively [10]. Coupled with this information, ES hemisuccinate (HS) acts as a ligand to selectively deliver chemotherapeutic agents in breast cancer cells. This offers a unique opportunity to customize ES/estrogen-conjugated drug-loaded nanovesicles for targeting ERs in breast cancer cells. In this study, we conjugated gelatin to the activated 30 -HS form of ES to achieve targeted lycopene delivery to breast cancer cells. Gelatin, an FDA-approved polymeric coating agent, was used for lycopene encapsulation because of its biocompatibility and wider acceptance. Here, we report the desiconjugated gelatin nanoparticles, fabrication, optimization, and characterization of estrone-conjugated lycopene-loaded gelatin nanoparticles (Lycopene-ES-conjugated gelatin nanoparticles) for targeting ERs and examine their potential for drug delivery in vitro[11].

2. MATERIAL AND METHODS

Lycopene was extracted from Tomatoes procured from the market and after washing were air-dried. Tomatoes were extracted out using petroleum ether for at least 30 minutes using mixers. Then the extract was filtered with filter paper, the solvent was separated in vacuum by rotary evaporator at 40°C. gelatin , dimethyl sulfoxide, MTT, glutaraldehyde, 1-(3-dimethyl-aminopropyl)-3-ethyl-carbodimide hydrochloride, N-hydroxysuccinimide, 2,4,6-trinitrobenzene sulfonic acid (TNBS), and ES were from Sigma Aldrich Pvt Ltd. All common reagents and solvents were of the highest analytical grade.

Preparation and evaluation of lycopene-conjugated gelatin nanoparticles and gelatin alone bearing lycopene

Lycopene was loaded onto ES-conjugated gelatin and gelatin alone to compare the kinetics of drug release and cytotoxic activity. Ten formulations of nanoparticles were prepared using the two-step desolvation method [12]. Briefly, 25 ml of a 5% w/v gelatin solution was prepared at room temperature. Gelatin was desolvated by adding 25 ml of acetone or ethanol dropwise and the mixture was left for sedimentation. The supernatant was discarded and the sediment was redissolved in 25 ml of water at varying pH ranging from 2 to 12 with 50 mg of lycopene. Acetone or ethanol was again added dropwise. These conjugated gelatin nanoparticles were further cross-linked with GLA (25% aqueous solution), excess of which was neutralized by adding 500 mg of glycine [13]. Purification was performed by centrifugation at 8000g and the desolvating agent was removed by slow vaporization over 24 h. Fluorescein isothiocyanate (FITC) was labeled to a lycopene appended formulation

3. RESULTS

Characterization of nanoparticles

Particle size and surface charge analysis

The mean particle size and surface charge (B) of Lycopene-conjugated gelatin nanoparticles and lycopene-es-conjugated gelatin nanoparticles were determined using a Zeta-Sizer .

Encapsulation efficiency and drug-loading capacity

The encapsulation efficiency of all the formulations, was calculated by dispersing the nanoparticles (50 mg) in 50 ml of 0.02 N hydrochloric acid, followed by warming for a few minutes, incubation for 48 h, and centrifugation at 8000g. The supernatant was filtered through a 0.2 mm membrane filter and an aliquot of the filtrate was diluted appropriately with the respective solvent system. The concentration of Lycopene in all the formulations was determined by measuring the optical density at 311.2 nm using a UV–Visible Spectrophotometer (Shimadzu, Kyoto, Japan) [8].

The encapsulation efficiency and drug-loading capacity were calculated using the following formula:

Encapsulation efficiency=Amount of drug entrapped/Amount of drug added*100

Drug loading capacity=Amount of drug present/Practical yield of nanoparticles*100

Transmission electron microscopy (TEM)

Particle shape and morphology of nanoparticles were determined using TEM at a voltage of 80 kV. (Fig 1)

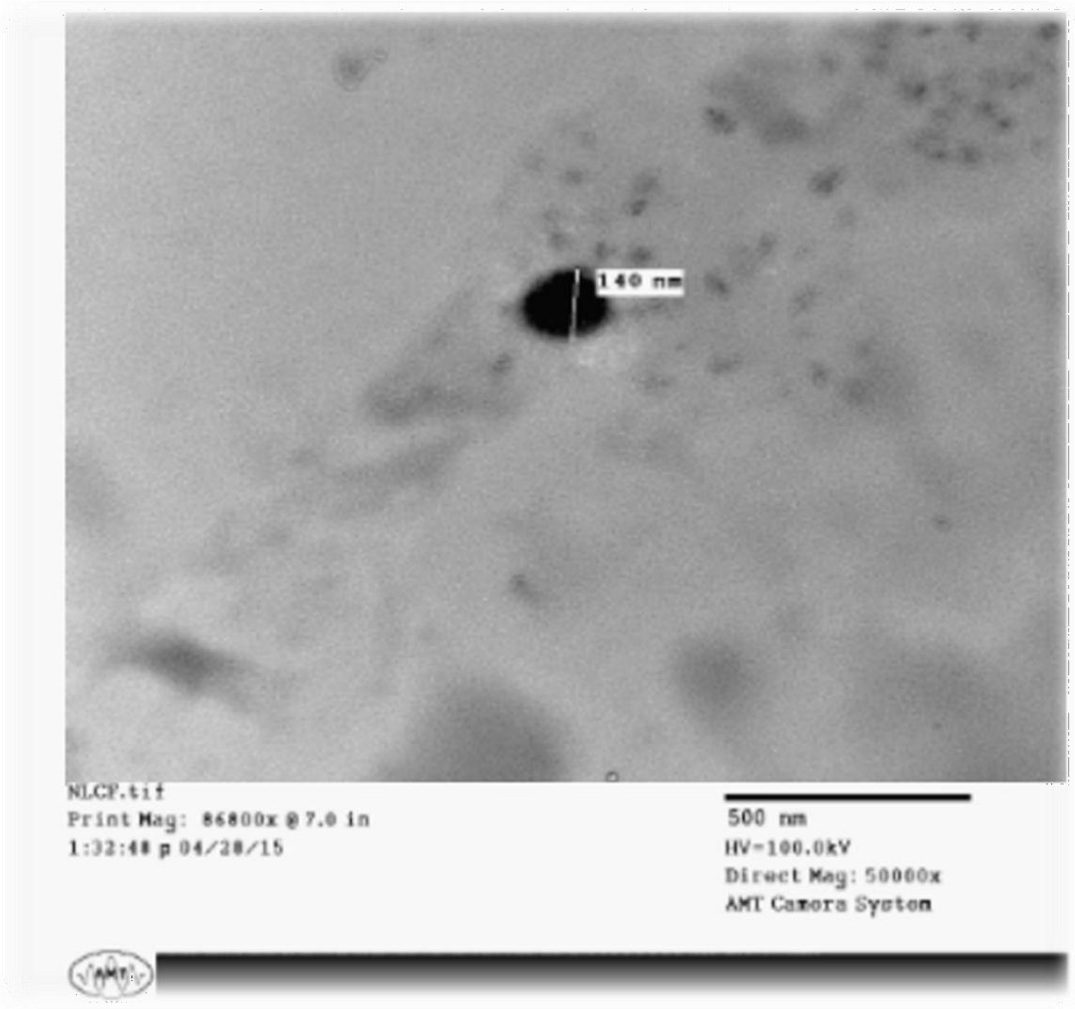

Fig 1:TEM image of optimized formulation

In-vitro drug-release kinetics

Dialysis was used to determine the drug-release kinetics of the nanoparticles . Briefly, 2 ml of Lycopene-conjugated gelatin nanoparticles and lycopene-es-conjugated gelatin nanoparticles were placed in dialysis bags and dialyzed against 250 ml of PBS (10 mmol/l, pH 4.5) and PBS (10 mmol/l, pH 7.4) maintained at 37 ^{0}C with a rotation speed of 50 rpm, followed by withdrawal of 5 ml of sample at different time intervals. The samples were further replaced with fresh buffer of the same pH to mimic sink conditions. The Lycopene concentration in the sample was determined by measuring its optical density using a UV–Visible Spectrophotometer (Shimadzu) [8]. The extent of drug release was calculated using a mathematical model based on zero-order or first-order kinetic release of drug from colloidal matrices [14]. The release kinetics was calculated using the following mathematical model (In[Qm0 – C1(V1+V2)]=In QmKCVt)

where C1 is the concentration of drug in the dialysate (outer solution);
Qm 0 is the total amount of drug associated with the nanoparticles at time zero; Km is the first-order release rate constant; KC is the apparent permeability constant of dialysis tubing; and V1 and V2 are the volumes of solution outside and inside the dialysis bag. VT = V1+ V2 and KCV = KCVT/V1V2

Effect of process parameters on particle size and entrapment efficiency of GN

Samples	pH	Temperature(^{0} C)	Glutaraldehyde(Microlitres)	Size (nm)	Polydispersity index	Encapsulation efficiency	Drug-loading capacity (mg)/10 mg	Zeta Potential
Lyc-GN	2.8	40	200	122±20	0.096±0.011	86.1±2.1	9.16	–42.1±0.5
Lyc-GN1	2.8	40	200	139±31	0.071±0.009	63.3±3.3	-	-
Lyc-GN2	2.8	40	200	160±25	0.065±0.0011	59.4±2.1	-	-
Lyc-GN3	7.6	40	200	190±28	0.091±0.040	38.9±6.9	-	-
Lyc-GN4	4.7	40	200	220±33	0.013±0.040	22.8±1.9	-	-
Lyc-GN5	2.88	40	200	170±26	0.029±0.098	30.9±3.5	-	-
Lyc-GN6	2.8	40	200	190±22	0.060±0.032	36.5±2.4	-	-
Lyc-GN7	2.8	40	400	180±34	0.077±0.062	40.9±9.7	-	-
Lyc-GN8	2.8	40	500	230±34	0.047±0.032	25.9±3.4	-	-
Lyc-GN9	2.8	40	200	250±45	0.057±0.063	38.9±6.9	-	-
Lyc-ES-GN	2.8	40	200	165±25	0.061±0.070	85±2.9	9.22	–31.1±0.8

In vitro release rate constants

Samples	Entrapment efficiency	Release rate constants Km1, Km2, Km (mg/h)	Initial drug release (%)	Terminal drug release (%)	Total drug release (%)
Lyc-GN	86.1±2.1	2.92*10–3, $1.27*10^{-3}$, 3.30*10–3	49.2	45.8	95.0
Lyc-ES-GN	85±2.9	$2.80*10^{-3}$, $1.34*10^{-3}$, $2.86*10^{-3}$	42.3	43.4	85.7

Km_1, initial rate constant (release of entrapped + free drug); Km_2, terminal rate constant (release of entrapped drug); Km, $Km_1 – Km_2$ (rate constant for the release of free drug).

4. DISCUSSION

Drug -release efficacy of Lycopene-ES-GN targeted to breast cancer MCF-7 cells. These novel nanoparticles were designed, synthesized, and characterized to achieve better targeting of lycopene to the ERs selectively overexpressed on breast cancer cells when tumors are hormone responsive [9]. We have optimized formulations taking into consideration the effects of pH, temperature, concentration of GLA, desolvating agents, and entrapment efficiency on nanoparticle size. During the course of experimentation, we found that changes in pH and temperature influenced the nanoparticle size.

5. CONCLUSION

Nanoparticle preparation showed that Lyc-GN and Lyc-ES-GN can be synthesized with a narrow particle size distribution. In addition,the use of acetone or ethanol as a desolvating agent during the fabrication of nanoparticles was crucial to control the size of nanoparticles to achieve better cellular targeting.

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