# siRNA Delivery Using Biodegradable Nanoparticles for Breast Cancer Therapy

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## ABSTRACT

Biodegradable nanoparticles are generally described as sub-cellular colloidal particles, composed of natural or synthetic polymers, varying in size between 10 – 1000 nm. A nanoparticle-based drug delivery system usually aims to help reduce side-effects of various harmful drugs by decreasing their systemic levels, and increasing the drug availability at the target site. Polyethylenimine (PEI)-coated human serum albumin (HSA) nanoparticles form suitable biocompatible carriers for therapeutic molecules. Small interfering RNA (siRNA) molecules have suggested great promise as a future therapy for breast cancer. In order to overcome the problems of degradation and low cellular transfection associated with siRNA, PEI-HSA nanoparticles are used. These nanoparticles are characterized to allow efficient delivery of siRNA to MCF-7 breast cancer cells. A satisfactory level of transfection indicates the potential of using PEI-HSA nanoparticles as a means to carry out gene therapy.

***Keywords***: nanoparticles, gene silencing, drug delivery, breast cancer

## 1 INTRODUCTION

Nanoparticles, as the basis of various drug delivery systems, have been intensively studied over the past decade or so. Polymeric Nanoparticles can be defined as sub-cellular colloidal particles, composed of natural or synthetic polymers {Hans, 2002 1 /id}. Nanoparticles may help to stabilize the compound to be delivered and increase their half-life by protecting it from hydrolysis or epimerization, solubilize certain therapeutics for intravascular administration, and may also allow for controlled delivery {Fonseca, 2002 2 /id}. Nanoparticles may vary in size between 10 nm to 1000 nm. Due to their sub-cellular sizes, nanoparticles can easily pass through the thin capillary beds into tissues, allowing delivery of the therapeutic compound to different tissues of the body {Gaumet, 2008 3 /id}. As nanoparticles can be taken up by the cells, the therapeutics they deliver may have an intracellular target, such as the mitochondria, nucleus, or the cytoplasm {Panyam, 2003 4 /id}.

It is important to assess the biocompatibility and biodegradability of the polymer or material that the nanoparticles are composed of, because the remnants of the nanoparticles must be degraded and metabolized after unloading the cargo. Ideally, the nanoparticles should be broken down into non-toxic material that is easily cleared from circulation in order to avoid any toxic effects.

Part of the immense interest in polymeric nanoparticles stems from the fact that they have potential for a wide range of applications. Cancer drugs, most of which have very harmful side-effects, can be delivered in a targeted manner using nanoparticles. Tumour tissue has impaired function and increased permeability, due to which nanoparticles can accumulate within cancerous cells more easily and deliver drugs {Liu, 2007 5 /id}.

Breast cancer leads to an astounding number of deaths globally among women and thus, various kinds of therapies are being devised to fight breast cancer. Small interfering RNA (siRNA) has shown potential for developing into an effective cancer therapeutic. siRNA leads to the specific degradation of the messenger RNA molecule of a certain gene, preventing its expression. The blockage of specific gene expression by siRNA drives interest in its application for cancer therapy {Bumcrot, 2006 6 /id}. However, therapeutic function of siRNA is limited due to its rapid degradation and low cellular uptake.

In the current study, we employ a nanoparticle-based delivery system to carry siRNA into tumor cells. The aim of this study is to increase cellular internalization of siRNA and improve its stability.

## 2 METHODS

### 2.1 Formation of HSA nanoparticles

HSA particles were synthesized at room temperature using a desolvation technique with 40 mg/ml HSA. 40 mg HSA was added to 1 ml of 10 mM NaCl (aq) under constant stirring. Upon complete dissolution, the solution was titrated to pH 8.5-9 with 1 N NaOH (aq). This aqueous phase was subjected to desolvation by the slow drop-wise addition of ethanol to the continuously stirred HSA solution until the solution became turbid. Lastly, PEI was added to the newly formed particles. PEI was allowed to coat the HSA nanoparticles under constant stirring for 6 hrs. In order to synthesize siRNA-loaded HSA nanoparticles, the specified amount of siRNA was added to 1 ml of HSA solution after adjusting the pH and stirred for 30 min., followed by ethanol addition.

## 2.2 Purification of nanoparticles

The newly formed HSA nanoparticles were subjected to ultra-sonication for 10 min. Firstly, ultra-centrifugation was carried out at 16000 g for 15 min, followed by the second round of ultra-centrifugation of the supernatant of the first at 17000 g for 25 min. The supernatant of the second round was then ultra-centrifuged at 18000 g for 30 min. The pellets from all three rounds of centrifugation were re-dispersed in 10 mM NaCl (aq) to the original volume and ultra-sonicated.

## 2.3 Particle characterization

The particle size, zeta potential and polydispersity index (PDI) were measured with electrophoretic laser Doppler anemometry, using a Zeta Potential Analyzer. The shape and size of the nanoparticles was observed by transmission electron microscopy (TEM).

## 2.4 Transfection of nanoparticles

MCF-7 breast cancer cells were grown in Dulbecco's modified Eagle's Medium (DMEM) with 10% Fetal Bovine Serum (FBS). Cells were cultured in a humidified incubator containing 5% $CO_2$ at 37˚C. Before transfection, cells were washed with Phosphate buffered saline (PBS) and replenished with fresh DMEM without FBS. The empty and siRNA-loaded PEI-coated HSA nanoparticles were added to the cells and incubated at 37˚C. After 4 hrs of incubation of cells with the nanoparticles, the culture medium was replaced with fresh DMEM, containing 10% FBS. Under the fluorescence microscope (TE2000-U, Nikon; USA), pictures were taken to assess the levels of transfection.

# 3 RESULTS

Using the ethanol desolvation technique, conditions were optimized to achieve an approximately 90 nm size of HSA nanoparticles (Figure 1), with a polydispersity index of $\leq$ 0.30. Nanoparticle were prepared using HSA concentration of 20 mg/ml, with and without loading siRNA, resulting in well-dispersed nanoparticles with an irregular shape. Figure 1 shows a TEM image of the prepared nanoparticles loaded with siRNA.

Increasing quantities of PEI were added per mg of HSA, which illustrated a decreasing nanoparticle size until 6.25 µg of PEI. The surface charge of the nanoparticles was recorded to be +26 mV. Similarly, increasing the amount of PEI for coating resulted in a more positive surface charge, as shown by the zeta potential measurements (data not shown). The concentration of HSA showed little or no effect on particle size or zeta potential of the particles. The effect of the pH of the HSA solution on particle characteristics were also tested. As the pH of the HSA solution became more acidic, the zeta potential resulted in more positive charge. A more basic pH of the HSA solution resulted in smaller sized particles.

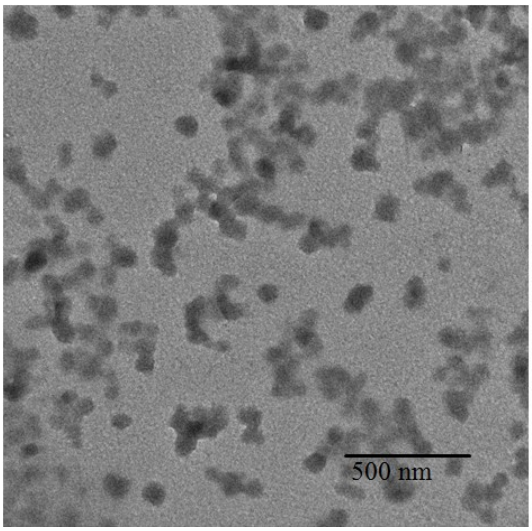


Figure 1: TEM image of siRNA-loaded PEI-coated HSA nanoparticles

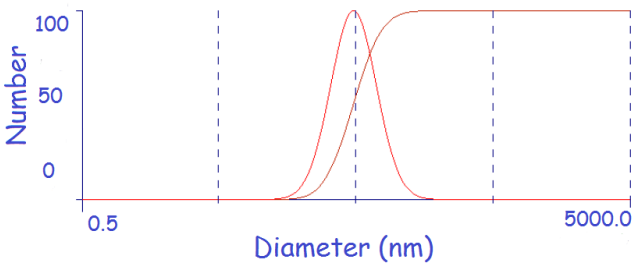


Figure 2: siRNA-loaded PEI-coated HSA particles showed a narrow size distribution with an average of ~90 nm.

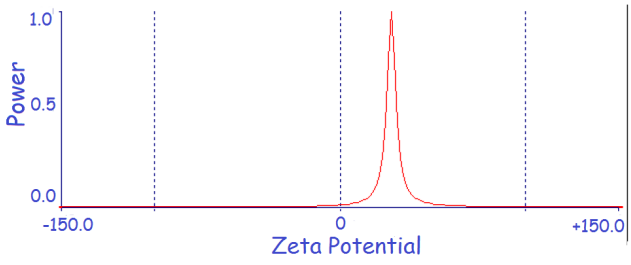


Figure 3: siRNA-loaded PEI-coated HSA nanoparticles coated with 6.25 µg of PEI.

The transfection efficiency of PEI-coated HSA nanoparticles into MCF-7 cells was assessed by synthesizing the nanoparticles with HSA tagged with Fluorescein isothiocyanate (FITC). After incubating MCF-7 cells that had been seeded in a 96 well-plate with FITC-HSA nanoparticles, the cells were washed with PBS thrice

and then viewed under the fluorescence microscope. Figure 3 illustrates MCF-7 cells transfected with PEI-coated FITC-HSA nanoparticles.

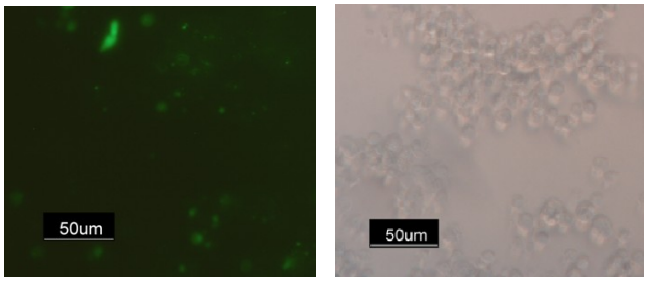


Figure 4: siRNA-loaded FITC-HSA nanoparticles internalized by MCF-7 cells.

## 4 DISCUSSION

Non-viral delivery systems are being studied as they hold promise of overcoming the issues related to viral delivery vectors. Adeno- or retroviruses impose risks of immunogenicity and pathogenicity, hindering their clinical application. On the other hand, nanoparticles also possess the advantages of easy administration, biocompatibility and a possibility of targeted delivery using nanoparticles [7].

As HSA is found in the blood in abundance, it is unlikely to cause any immunogenic or cytotoxic effect when administered in the form of nanoparticles. PEI is incorporated as a stabilizing agent to increase the circulation time of the HSA nanoparticles. In addition, PEI also provides the nanoparticles with a positive surface charge, resulting in higher levels of transfection.

Using nanoparticles to carry and deliver siRNA seems like a viable solution to the issue of extremely rapid degradation of siRNA. However, further studies are required before any conclusion can be made. In vivo studies should also be carried out to determine whether this nanoparticle delivery system is able to remain in circulation long enough to deliver the cargo of interest.

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