

Biodistribution and Pharmacokinetic Analysis of Long-Circulating Thiolated Gelatin Nanoparticles Following Systemic Administration in Breast Cancer-Bearing Mice

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Received 16 March 2006; revised 3 August 2006; accepted 26 September 2006

Published online in Wiley InterScience (www.interscience.wiley.com). DOI 10.1002/jps.20813

ABSTRACT: The objective of the present study was to modify thiolated gelatin nanoparticles with poly(ethylene glycol) (PEG) chains and examine their long circulating and tumor-targeting properties *in vivo* in an orthotopic a human breast adenocarcinoma xenograft model. The crosslinked nanoparticle systems were characterized to have a size of 150–250 nm with rapid payload release properties in a highly reducing environment. Upon PEG modification, the nanoparticle size increased to 300–350 nm in diameter. The presence of PEG chains on the surface was confirmed by characterization with electron spectroscopy for chemical analysis. The *in vivo* long-circulating potential, biodistribution and passive tumor targeting of the controls, and PEG-modified thiolated gelatin nanoparticles were evaluated by injecting indium-111 (^{111}In)-labeled nanoparticles into breast tumor (MDA-MB-435)-bearing nude mice. Upon modification with PEG, the nanoparticles were found to have longer circulation times, with the plasma and tumor half-lives of 15.3 and 37.8 h, respectively. The results also showed preferential localization of thiolated nanoparticles in the tumor mass. The resulting nanoparticulate systems with long circulation properties could be used to target encapsulated drugs and genes to tumors passively by utilizing the enhanced permeability and retention effect of the tumor vasculature. © 2006 Wiley-Liss, Inc. and the American Pharmacists Association J Pharm Sci 96:397–407, 2007

Keywords: thiolated gelatin nanoparticles; poly(ethylene glycol); surface modification; long circulation time; passive tumor targeting; biodistribution

INTRODUCTION

With simultaneous advances in molecular biology and nanotechnology, the interdisciplinary field of nanomedicine is rapidly evolving.^{1,2} One of the areas of interest in nanomedicine is targeted delivery of molecular medicine to specific disease sites in the body. Nanoparticles including liposomes, micelles, and polymeric systems have revolutionized delivery of drugs and genes to

selective targets, including intracellular accumulation and delivery to cellular organelles.^{3–8} For targeted delivery to solid tumors *in vivo*, nanoparticles can accumulate at the site either by passive or active mechanisms. Passive tumor delivery is primarily achieved by the enhanced permeability of the tumor vasculature and lack of lymphatic drainage by a process known as the enhanced permeability and retention (EPR) effect.^{9–11} Because of their small size, ease of production and administration, and increased stability when compared to the other colloidal carriers, the polymeric nanoparticles are used as carriers of choice to deliver anticancer drugs and genes to tumors.

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Journal of Pharmaceutical Sciences, Vol. 96, 397–407 (2007)
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Systemically administered polymeric nanoparticles are rapidly cleared from the circulation by a process of opsonization, which is initiated by complement activation and preferential uptake of the nanoparticles by the macrophages of the reticuloendothelial system (RES). For delivery to solid tumor, surface modification of polymeric nanoparticles with water-soluble polymers, such as poly(ethylene glycol) (PEG), affords long circulation time and passive targeting potential to the tumor mass. Surface-bound PEG chains prevent protein binding, complement activation, and preferential uptake by the RES through the steric repulsion mechanism. The PEG-modified nanoparticles are then taken up by the tumor mass by the EPR effect. In a review article, Moghimi et al.¹² have extensively discussed the development and applications of long-circulating and target-specific nanoparticles.

In a previous study,⁷ we have described the synthesis of thiolated gelatin using type B gelatin and 2-iminothiolane, preparation of nanoparticles, and cytosolic DNA delivery potential in response to higher intracellular glutathione concentrations. Qualitative and quantitative analysis of transfection in NIH-3T3 murine fibroblast cells by the nanoparticles carrying plasmid DNA encoding for enhanced green fluorescent protein (EGFP-N1) was done by fluorescence microscopy and fluorescence-activated cell sorting (FACS). Qualitative results showed highly efficient expression of green fluorescence protein in the crosslinked thiolated nanoparticulate systems that remained stable for up to 96 h. Quantitative results from FACS showed that the crosslinked, thiolated gelatin nanoparticles were effective in transfecting NIH-3T3 cells than other nanocarrier systems examined, including Lipofectin[®], a commercially-available cationic lipid transfection reagent. The high transfection efficiency associated with the thiolated gelatin nanoparticles could be attributed to the increased stability from additional cross-linking, noncomplexed DNA delivery system, trigger release of the payload in a reducing environment inside the cell, and reduced cytotoxicity. The results clearly showed that thiolated gelatin nanoparticles were effective in intracellular plasmid DNA delivery and afforded an efficient transfection system.

Despite numerous advantages of thiolated gelatin as a biocompatible, biodegradable, and noncondensing polymeric matrix for intracellular DNA delivery, it is still necessary to chemically modify the nanoparticles so that it has longer

circulation time in the physiological environment that can lead to efficient accumulation at the tumor site and internalization in cells. There are various factors that influence the extent or degree of long circulation, these include—nature of the polymer used, length of the hydrophobic anchor, polymer molecular weight (i.e., chain length and energy of molecular motion), and the quantity of the protective polymer on the particle surface. The size, molecular weight, and shape of the PEG fraction and the linkage used to connect it to the entity of interest determine the consequences of PEG modification, in relation to protein adsorption and pharmacokinetic properties such as volume of distribution, circulation time, and renal clearance.

In the present study, we have investigated the biodistribution and tumor-targeting potential of the control and PEG-modified thiolated gelatin nanoparticles *in vivo* in estrogen-negative human breast adenocarcinoma (MDA-MB-435)-bearing athymic mice. Noncompartmental pharmacokinetic analysis was performed to compare the distribution parameters between the control and PEG-modified nanoparticles.

EXPERIMENTAL METHODS

Materials

Type-B gelatin (225 bloom strength) having 100–115 mmole of carboxylic acid/100 g of protein, an isoelectric point of 4.7–5.2, and an average molecular weight 40000–50000 Da was purchased from Sigma-Aldrich (St. Louis, MO). Methoxy-PEG-succinimidyl glutarate of molecular weight of 2000 Da was purchased from Sun Bio (Orinda, CA). Traut's reagent (2-iminothiolane) and Ellman's reagent [5,5'-dithio-bis-(2-nitrobenzoic acid)] were purchased from Pierce Biotechnology, Inc., (Rockford, IL). Absolute ethyl alcohol (200 proof, 99.5% ACS reagent) was obtained from Acros Chemicals (Pittsburgh, PA). Glyoxal 40% (w/v), glycine, HEPES, anhydride of diethylenetriaminepentaacetate (DTPA) sodium citrate, and sodium chloride were purchased from Fisher Scientific (Fair Lawn, NJ). The radiolabeling agent indium-111 chloride was purchased from Perkin Elmer (Wellesley, MA). The estrogen-negative human breast adenocarcinoma cell line (MDA-MB-435) was obtained from American Type Culture Collection (ATCC, Manassas, VA) and maintained in Roswell Park Memorial Institute

(RPMI)-1640[®] with L-glutamine, supplemented with 10% fetal bovine serum and 1% of penicillin-streptomycin. All aqueous solutions and reagents were prepared in deionized distilled water from Nanopure II (Barnstead/Thermolyne, Dubuque, IA).

Preparation of Thiolated Gelatin Nanoparticles

Thiolated gelatin was synthesized as reported previously.⁷ Briefly, thiolated gelatin was prepared by covalent modification of the primary amino groups of type-B gelatin by the addition of sulfahydril moieties. For the synthesis, 1 g of gelatin was dissolved in 100 mL of deionized water and then incubated with varying amount of 2-iminothiolane hydrochloride (up to 100 mg/g) at room temperature for 15 h. Any unreacted 2-iminothiolane was removed by repeated dialysis against 5 mM HCl, followed by 1 mM HCl solution for 24 h each. The purified thiolated gelatin was dried *in vacuo* and stored at -80°C for further use.

Based on initial cytotoxicity and transfection results,⁷ the nanoparticles for these studies were prepared with thiolated gelatin that was formed by reaction of 20 mg of 2-iminothiolane/g of type-B gelatin, which has sulfahydril groups equivalent to 6.1 ± 0.7 mmole/g biopolymer. The nanoparticles were prepared with 1% (w/v) aqueous solution of thiolated gelatin in a temperature-controlled water bath at 37°C . The pH of the resulting solution was adjusted to 7.0 with 0.2 M sodium hydroxide. The nanoparticles were formed when the solvent composition was changed from 100% water to 75% by volume of hydro-alcoholic solution, upon gradual addition of absolute ethanol under continuous stirring conditions. The formed nanoparticles were further crosslinked with 0.1 mL of 40% (v/v) aqueous solution of glyoxal for the desired time interval and any unreacted aldehyde residues were quenched with 0.2 M glycine solution. The particles obtained were centrifuged at 16000 rpm for 30 min and the pellet was washed twice with deionized distilled water. The purified nanoparticles were freeze-dried and stored at room temperature.

Surface Modification of Nanoparticles with PEG

The control gelatin (Gel) and thiolated gelatin (SHGel) nanoparticles collected after centrifugation were suspended in 0.1 M phosphate buffer (pH 7.4), and incubated with five times molar

excess (2 mg of PEG/mg of gelatin or thiolated gelatin nanoparticles) of methoxy-PEG-succinimidyl glutarate (2000 Da) for 2 h at room temperature. At the end of the reaction, the nanoparticles were collected by centrifugation and assayed for the degree of PEG modification by using trinitrobenzene sulfonic acid (TNBS) assay.¹³ In the TNBS method, the number of free amino groups is estimated by a colorimetric reaction that results in the formation of a yellow-colored product, which shows maximum absorbance at 420 nm. The gelatin and thiolated gelatin (control) nanoparticles, and the PEG-conjugated gelatin and thiolated gelatin nanoparticles were dispersed in pH 8.5 alkaline borate buffer, and allowed to react with the TNBS reagent at room temperature. The reaction mixture was centrifuged at 5000 rpm for 5 min, and the absorbance of the supernatant solution was measured at 420 nm using a Shimadzu UV160U spectrophotometer (Columbia, MD). Using this assay, the percentage of surface-accessible amine groups that could react with TNBS was calculated. Since methoxy-PEG-succinimidyl glutarate reacts with the primary amine residues in gelatin and thiolated gelatin, the degree of PEG modification was estimated indirectly, based on differences between the absorbance values of the supernatant from the control (gelatin and thiolated gelatin nanoparticles) and after PEG modification.

Characterization of PEG-Modified Nanoparticles

Particle Size Analysis

The mean particle sizes and size distribution of unmodified and PEG-modified nanoparticles of gelatin (PEG-Gel) and thiolated gelatin (PEG-SHGel) were analyzed using ZetaPALS, 90Plus (Brookhaven Instruments Corporation, Holtsville, NY). The colloidal suspension of the nanoparticles was diluted with deionized distilled water and the particle size analysis was carried out at a scattering angle of 90° and at 25°C .

Surface Charge Measurements

Zeta potential (ζ) measurements of the unmodified and PEG-modified nanoparticles of gelatin and thiolated gelatin in suspension in deionized water were performed using a ZetaPALS (Brookhaven Instruments Corporation). The nanoparticles were dispersed in deionized water

and the zeta potential values were measured at the default parameters of dielectric constant, refractive index, and viscosity of water. The average zeta potential values were computed, based on Smoluchowski equation.

Scanning Electron Microscopy (SEM)

Freeze-dried nanoparticle samples were mounted on an aluminum sample mount and sputter-coated with gold–palladium. The samples were then observed for surface morphology with a Hitachi 4800 field emission scanning electron microscope.

Electron Spectroscopy for Chemical Analysis (ESCA)

In order to determine the surface presence of PEG chains on the modified nanoparticles, freeze-dried formulations of the controls and PEG-modified nanoparticles were analyzed by ESCA. ESCA (or X-ray photoelectron spectroscopy) determines the elemental and chemical composition of the surface at up to 10 nm distance. ESCA was performed at the National ESCA and Surface Analysis Center for Biomedical Problems (NESAC/BIO), University of Washington (Seattle, WA). The samples were placed in an ultrahigh vacuum environment and exposed to a low-energy, X-rays beam, resulting in an emission of secondary photoelectrons from the surface of the nanoparticles. The energy of the electrons emitted is characteristic of the element and the molecular orbital from which it is emitted. Only those electrons that escape from the surface of the sample without loss of energy contribute to the signal peak. For each surface chemical element, a spectrum was obtained by plotting the number of detected electrons as a function of the binding energy (in millivolts). The surface elemental composition was determined using standard Scofield photoemission cross sections. High-resolution analysis of the C_{1s} spectra was performed to determine the exact chemical composition from the hydrocarbon (C–C or C–H at 285 eV), ether (C–O at 286.4 eV), and the carbonyl (C=O at 288.1 eV) envelopes, and the relative composition of each functionality was determined from the area under the peak. In PEG-modified nanoparticles, the surface presence of ethylene oxide residues would translate into increase in the ether signal in the C_{1s} spectra.¹⁴

Radiolabeling with ^{111}In

In order to determine the fate of the nanoparticles in various tissues in the body after intravenous administration, they were labeled with indium-111 (^{111}In). The controls (i.e., Gel and SHGel) and PEG-modified (i.e., PEG-Gel and PEG-SHGel) nanoparticles were dispersed at a concentration of 5 mg/mL in HEPES buffered saline (HBS, pH 7.5). To this suspension, anhydride DTPA (4 mg/mL), dissolved in dimethylsulfoxide, was added under constant stirring at room temperature. The nanocarrier–DTPA mixture was incubated at room temperature for 1 h and the excess DTPA was removed by centrifugation at 7000 rpm for 10 min. The nanoparticles were washed and centrifuged twice to ensure proper removal of free DTPA. The nanoparticles conjugated with DTPA were then labeled with ^{111}In .

To the DTPA-conjugated control and PEG-modified nanoparticles suspended in HBS, 1 M HEPES was added to prevent any change in pH. This was followed by the addition of sodium citrate and ^{111}In chloride. The mixture was incubated for 1 h at room temperature and any free radioactivity was removed by centrifugation. The radiolabeled nanoparticles were re-suspended and centrifuged repeatedly, and the supernatant was measured for any traces of free radioactivity using a gamma counter. The nanoparticles were considered stably labeled with ^{111}In , when the radioactivity in the supernatant reached a minimal value. The specific radioactivity in microCi/mg of nanoparticles was assessed, in order to estimate the amount of radio-labeled nanoparticles to be injected per animal.

In Vivo Biodistribution Studies

Culture Conditions for MDA-MB-435 Cells

MDA-MB-435 estrogen-negative human breast adenocarcinoma cell line derived from breast ductal carcinoma of a Caucasian female was used for growing tumors in athymic mice. These cells were grown as a monolayer in culture using RPMI-1640 supplemented with L-glutamine, Pen-strep, and fetal bovine serum at 37°C and 5% CO_2 . These cells were metastatic in origin and were used to grow tumor models in mice.

Animals for Biodistribution Studies

All of the experiments described below with the use of radioactive materials in athymic mice were approved by Northeastern University's

Institutional Animal Care and Use Committee as well as the Radiation Safety Committee within the Office of the Environmental Health and Safety. Six weeks old female *Nu/Nu* (athymic) mice, weighing approximately 20 g, purchased from Charles River Laboratories (Wilmington, MA), were used for the biodistribution study. Upon arrival, the animals were housed in the Division of Laboratory Animal Medicine at Northeastern University and supplied with sterile rodent pellets and water *ad libitum*. The animals were allowed to acclimate for at least 48 h prior to any experimentation.

Development of Tumor Model

An orthotopic tumor xenograft model was developed by subcutaneous injection of 2.0 million MDA-MB-435 cells in 200 μ L of normal saline in the mammary fat pad region of lightly anesthetized female *Nu/Nu* mice. The animals were monitored daily during tumor development by palpation of the injected region and by monitoring the weight and motility of the animals. When the tumor volume had reached approximately 100 mm³ after 10 days postinoculation, the animals were divided into groups for administration of the control and PEG-modified nanocarrier formulations.

Biodistribution Studies

The MDA-MB-435 breast adenocarcinoma-bearing mice were injected intravenously with 2 μ Ci dose of the radiolabeled (¹¹¹In) of Gel, SHGel, PEG-Gel, and PEG-SHGel nanoparticles, dispersed in 200 μ L of normal saline via the tail vein. The mice were sacrificed at specific time points of 1, 2, 4, 6, 12, 24, and 48 h after administration. Five animals at each time point were sacrificed by cervical dislocation following anesthetization. Blood, tumor, and highly perfused organs such as heart, liver, kidneys, spleen, and lungs were excised and the radioactivity accumulated in each of these tissues was determined with a Wallac 1470 WizardTM automatic gamma counter (Wallac, Inc., Gaithersburg, MD). The weight of each organ was measured and the corresponding radioactivity in that organ was recorded. The number of counts-per-minute (CPM values from gamma counter) was converted into microCuries of radioactivity. The concentration of nanoparticles in blood, tumor, and other tissues was expressed as percent of recovered dose per gram of fluid or tissue.

Noncompartmental Pharmacokinetic Analysis

In order to determine the pharmacokinetic parameters associated with the biodistribution and accumulation of the Gel, SHGel, PEG-Gel, and PEG-SHGel nanoparticles in breast cancer-bearing mice, the analyses were carried with plasma and tumor data. Win-NonLin[®] software (Pharsight Corp., Mountain View, CA) was used to determine distribution parameters, half-life, clearance, volume of distribution, and the area-under-the-curve (AUC) of the control and PEG-modified nanoparticles as a function of time.

RESULTS AND DISCUSSION

PEG Modification of the Nanoparticles

The free amino groups on the surface of gelatin and thiolated gelatin nanoparticles were considered as 100%. There was an increase in PEG modification of the surface amino groups of the nanoparticles with an increase in incubation time of methoxy-PEG-succinimidyl glutarate up to 2 h. At the end of this time period, about 90% of the surface-accessible amine groups were modified upon incubation with five times molar excess (i.e., 2 mg of PEG/mg of gelatin or thiolated gelatin nanoparticles) of methoxy-PEG-succinimidyl glutarate.

Characterization of the PEG-Modified Nanoparticles

The mean particle diameter and surface charge of the Gel, SHGel, PEG-Gel, and PEG-SHGel nanoparticles are shown in Table 1. For the controls gelatin and thiolated gelatin nanoparticles, the

Table 1. Characteristics of the Control and Poly(Ethylene Glycol) (PEG)-Modified Nanoparticles

Type of Nanoparticle	Properties of Nanoparticles	
	Particle Diameter (nm)	Zeta Potential (mV)
Gelatin nanoparticles	230 $\pm$ 11.5 ^a	-8.45 $\pm$ 0.45
PEG-modified gelatin nanoparticles	329 $\pm$ 17.7	-7.72 $\pm$ 0.88
Thiolated gelatin nanoparticles	244 $\pm$ 6.8	-9.36 $\pm$ 0.64
PEG-modified thiolated gelatin nanoparticles	302 $\pm$ 9.2	-6.67 $\pm$ 0.77

^aMean $\pm$ SD. (*n* = 5).

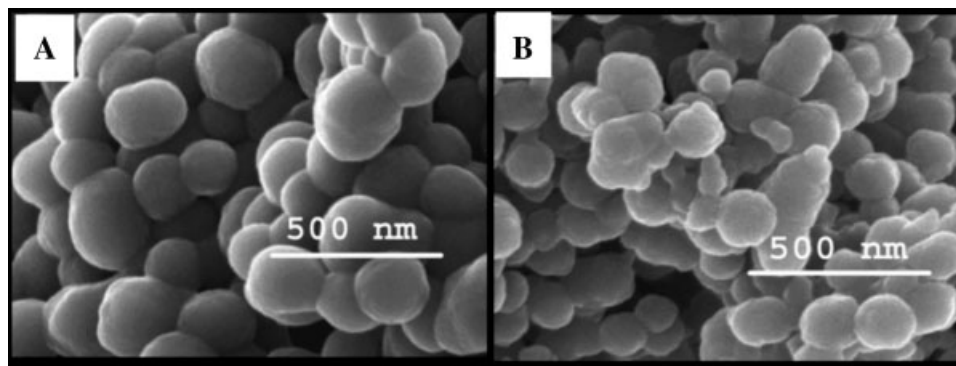


Figure 1. Scanning electron micrographs of gelatin (A) and thiolated gelatin (B) nanoparticles. The scale bar represents a distance of 500 nm.

average size was found to be in the range of 220–250 nm. However, owing to the surface modification, both the PEG-modified nanoparticles were found to have a larger particle size ranging from 290 to 350 nm. The increase in size of the nanoparticles clearly indicates the addition of PEG molecules on the surface of the nanoparticles, which results in the change in hydrodynamic diameter of the system in aqueous environment. Even with PEG modification, the nanoparticles will still have the size range below 400–600 nm that is necessary for accumulation in solid tumor by the EPR effect.^{15–18} When compared to other tumor models, the breast cancer tumors in the mammary fat pad region were found to have wide openings in the endothelial junctions ranging up to 4.5 μm .^{19,20} The controls gelatin and thiolated gelatin nanoparticles were found to have zeta potential values of -8.45 and -9.36 mV, respectively. In comparison, the PEG-modified gelatin and thiolated gelatin nanoparticles were found to have a surface charge of -7.72 and -6.67 mV, respectively. The slight decrease in the negative charge on the surface of PEG-modified nanoparticles could be due to the modification of the surface amino groups of the gelatin and thiolated

gelatin. SEM images in Figure 1 show that the gelatin and thiolated gelatin nanoparticles had spherical shape and smooth surface morphology.

The results in Table 2 show the peak intensities of the species C–H (hydrocarbon), C–O (ether), and C=O (carbonyl), obtained from $\text{C}_{1\text{S}}$ scan of the control and PEG-modified nanoparticles at their characteristic binding energies. The surface presence of PEG chains in the PEG-modified nanoparticles was confirmed by the increase in the relative peak intensities of ether linkage from the high-resolution spectra. The peak intensities of ether linkage in case of PEG-modified gelatin (PEG-Gel) were found to be 79.2% and PEG-modified thiolated gelatin (PEG-SHGel) nanoparticles resulted in 82.6%. In comparison, the gelatin and thiolated gelatin nanoparticles have shown ether peak intensities of 36 and 28.4%, respectively.

Plasma and Tumor Concentrations Versus Time Profiles

The plasma concentration-time profile of the Gel, SHGel, PEG-Gel, and PEG-SHGel nanoparticles was obtained by plotting percentage of recovered

Table 2. High-Resolution Electron Spectroscopy for Chemical Analysis $\text{C}_{1\text{S}}$ Peak Analysis for the Control and Poly(Ethylene Glycol) (PEG)-Modified Nanoparticle Surfaces*

Nanoparticle Type	Relative Peak Area (%)		
	C–H– (285.0 eV)	C–O– (286.3 eV)	C=O (288.1 eV)
Gelatin nanoparticles	33.5	36.0	30.4
PEG-modified gelatin nanoparticles	11.8	79.2	7.0
Thiolated gelatin nanoparticles	53.1	28.4	18.4
PEG-modified thiolated gelatin nanoparticles	11.9	82.6	5.5

*Electron spectroscopy for chemical analysis (ESCA) was performed at National ESCA and Surface Analysis Center for Biomedical Problems (NESAC/Bio), University of Washington, Seattle, WA.

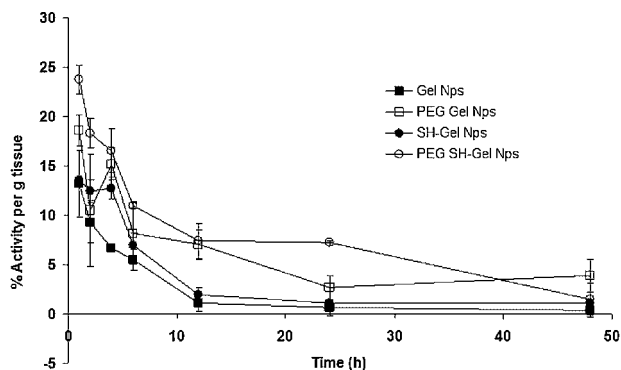


Figure 2. The change in nanoparticle concentrations as a function of time in the plasma. ^{111}In -labeled gelatin, thiolated gelatin, poly(ethylene glycol)-modified gelatin, and poly(ethylene glycol)-modified thiolated gelatin nanoparticles were administered intravenously to female *Nu/Nu* mice bearing MDA-MB-435 human breast adenocarcinoma xenograft.

radioactivity per gram of plasma sample, as a function of time and is represented in Figure 2. The plasma concentration-time profiles of the radiolabeled nanoparticles indicate a significantly greater recovery of the PEG-modified gelatin and thiolated gelatin nanoparticles, when compared to the controls. For instance, at the end of 6 h, about 11.0 and 8.2% of the dose was recovered in case of PEG-SHGel and PEG-Gel nanoparticles, as opposed to their unmodified nanoparticles that had 6.5 and 5.5%, respectively. At time points beyond 12 h, most of the dose of gelatin and thiolated gelatin nanoparticles was cleared off circulation with only 1.0–1.4% of the nanoparticles remaining, when compared to the PEG-modified nanoparticles that had retention of almost 7% of the dose.

Noncompartmental pharmacokinetic analysis was performed using Win-NonLin on the last

couple of data points of the four formulations and the pharmacokinetic parameters were reported in Table 3. There was no significant difference between Gel and SHGel nanoparticles with respect to elimination half-life (~ 3 h), mean residence time, maximum concentration in the blood, volume of distribution, and the rate of *in vivo* clearance. However, upon surface modification, the PEG-SHGel nanoparticles were found to have a half-life of 15.34 h, when compared to those of PEG-Gel with a half-life 10.69 h. In addition, the significantly high values of AUC associated with PEG-modified nanoparticles (threefold increase) indicate an increase in the extent and availability of the nanoparticles in circulation. The increase in circulation times of these PEG-modified nanoparticles could be explained by the reduced uptake by the RES. The technique of postmodification by PEG on the surface of the nanoparticles has successfully resulted in particles with steric hindrance that could reduce complement activation and subsequent uptake by the RES.

Upon modification with PEG, the ^{111}In -labeled nanoparticles resulted in a greater accumulation in the tumor, as seen in tumor concentration versus time profile in Figure 3. About 13% of the recovered dose of PEG-SHGel and PEG-Gel nanoparticles were retained in the tumor at 12 h time point, in contrast to Gel and SHGel nanoparticles that had 7 and 9% retention, respectively (Fig. 3). These values, again, are clearly indicative of the stealth properties of PEG-modified nanoparticles that resulted in reduced uptake by the RES and enhanced accumulation and retention in the tumor. The pharmacokinetic parameters calculated from the tumor data show an increased half-life for the PEG-SHGel nanoparticles of up to 37 h, followed by PEG-Gel nanoparticles of 34 h (Tab. 4). Among the unmodified nanoparticles, the SHGel

Table 3. Summary of Noncompartmental Pharmacokinetic Parameters of Control and Poly(ethylene Glycol) (PEG)-Modified Gelatin Nanoparticles in Plasma*

Pharmacokinetic Parameter	Gelatin Nanoparticles	PEG-Gelatin Nanoparticles	Thiolated Gelatin Nanoparticles	PEG-Modified Thiolated Gelatin Nanoparticles
Half-life (h)	2.94 ± 0.07^a	10.7 ± 0.08	2.98 ± 0.01	15.3 ± 0.03
Elimination rate constant (h^{-1})	0.23 ± 0.09	0.06 ± 0.00	0.23 ± 0.00	0.04 ± 0.00
Total body clearance ($\% \text{ h}/(\%/\text{g})$)	0.99 ± 0.00	0.30 ± 0.01	0.67 ± 0.01	0.25 ± 0.00
Volume of distribution ($\%(\%/\text{g})$)	9.50 ± 0.02	7.49 ± 0.06	8.70 ± 0.07	4.98 ± 0.04
Area-under-the-curve ($\% \text{ h/g}$)	97.8 ± 4.32	316.8 ± 23.4	142.2 ± 11.0	399.3 ± 8.61

* ^{111}In -labeled control and PEG-modified nanoparticles were injected in MDA-MB-231 human breast adenocarcinoma-bearing female *Nu/Nu* athymic mice.

^aMean $\pm$ SD. ($n = 4$).

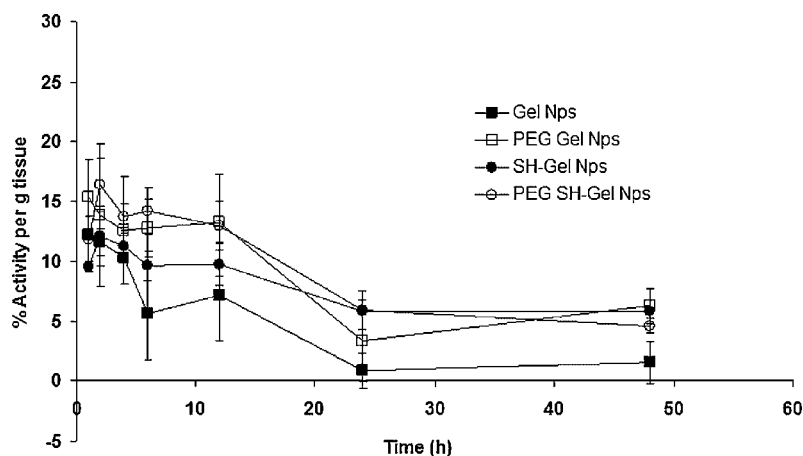


Figure 3. The change in nanoparticle concentrations as a function of time in the tumor mass. ^{111}In -labeled gelatin, thiolated gelatin, poly(ethylene glycol)-modified gelatin, and poly(ethylene glycol)-modified thiolated gelatin nanoparticles were administered intravenously to female *Nu/Nu* mice bearing MDA-MB-435 human breast adenocarcinoma xenograft.

nanoparticles had a tumor half-life of 28.98 h, when compared to the gelatin nanoparticles with a half-life of 18.51 h. Even though the thiolated gelatin nanoparticles had a low plasma half-life (3 h), they had a significantly higher retention half-life in the tumor mass and higher AUC values, indicating preferential uptake of the thiolated gelatin nanoparticles by the tumor cells (Tab. 4). The prolonged circulation and preferential uptake resulted in an enhanced ability of the PEG-modified thiolated gelatin (PEG-SHGel) nanoparticles to extravasate into tumors with leaky vasculature.

Biodistribution in Other Highly Perfused Organs

Upon injection of ^{111}In -labeled nanoparticles through tail vein, a major fraction of the dose up

to 45% in case of gelatin and 20–35% in case of PEG-modified gelatin (PEG-Gel) and thiolated gelatin (PEG-SHGel) nanoparticles was found in the liver, due to the recognition and uptake of the nanoparticles by the macrophages (Fig. 4). Localization of these nanoparticulate formulations in liver at time points as early as 1 h shows the instantaneous recognition of colloidal particles by the RES. However, it is the difference in distribution of the PEG-modified nanoparticles in the liver that has resulted in their longer circulation and availability in plasma. The decrease in uptake of the PEG-modified nanoparticles by the liver is, in fact, an indicative of the stealth properties of PEG which resulted in reduced uptake by the RES. The fraction of the dose that evades the RES has remained in circulation in the blood, leading to extravasation into leaky

Table 4. Summary of Noncompartmental Pharmacokinetic Parameters of Control and Poly(Ethylene Glycol) (PEG)-Modified Gelatin Nanoparticles in Tumor Mass*

PK Parameter	Gelatin Nanoparticles	PEG-Gelatin Nanoparticles	Thiolated Gelatin Nanoparticles	PEG-Modified Thiolated Gelatin Nanoparticles
Half-life (h)	18.5 ± 0.06^a	34.2 ± 1.04	29.0 ± 1.22	37.8 ± 0.82
Elimination rate constant (h^{-1})	0.03 ± 0.00	0.02 ± 0.00	0.02 ± 0.00	0.02 ± 0.00
Total body clearance ($\% \text{ h}/(\%/\text{g})$)	0.45 ± 0.01	0.14 ± 0.00	0.19 ± 0.00	0.15 ± 0.00
Volume of distribution ($\% / (\%/\text{g})$)	11.3 ± 0.21	8.02 ± 0.11	8.59 ± 0.09	7.62 ± 0.08
Area-under-the-curve ($\% \text{ h}/\text{g}$)	204.2 ± 19.9	668.3 ± 34.4	495.4 ± 8.46	642.8 ± 17.0

* ^{111}In -labeled control and PEG-modified nanoparticles were injected in MDA-MB-231 human breast adenocarcinoma-bearing female *Nu/Nu* athymic mice.

^aMean $\pm$ SD ($n = 4$).

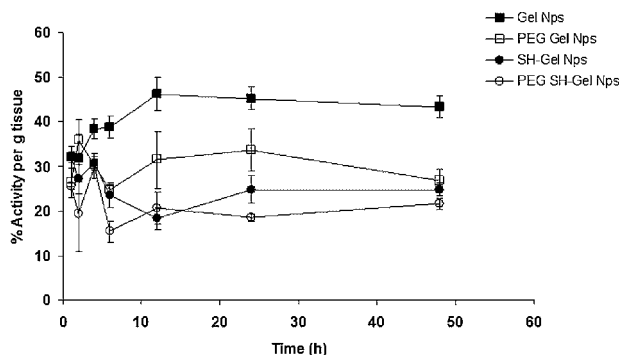


Figure 4. The change in nanoparticle concentrations as a function of time in the liver. ^{111}In -labeled gelatin, thiolated gelatin, poly(ethylene glycol)-modified gelatin, and poly(ethylene glycol)-modified thiolated gelatin nanoparticles were administered intravenously to female *Nu/Nu* mice bearing MDA-MB-435 human breast adenocarcinoma xenograft.

vasculature of the tumors which is achieved by the EPR effect.

Besides the plasma, tumor, and liver, the nanoparticles were predominantly found in the kidneys and spleen and, to a lesser extent, in the lungs and heart. Results in Figure 5 show the disposition of the nanoparticles in spleen, kidney, lungs, and heart for various time points up to 48 h. A progressive increase in the radioactivity in the kidneys was observed at later time points, indicating the disintegration and ultimately the elimination of the ^{111}In -labeled nanoparticles from the systemic circulation. Upon enzymatic degradation of the Gel, SHGel, PEG-Gel, and PEG-SHGel nanoparticles, the resulting low molecular weight products are water soluble and would be excreted by the renal route. In addition, the thiolation was shown to result in twofold increase in accumulation in the spleen, when compared to gelatin nanoparticles (both unmodified and PEG-modified). Almost,

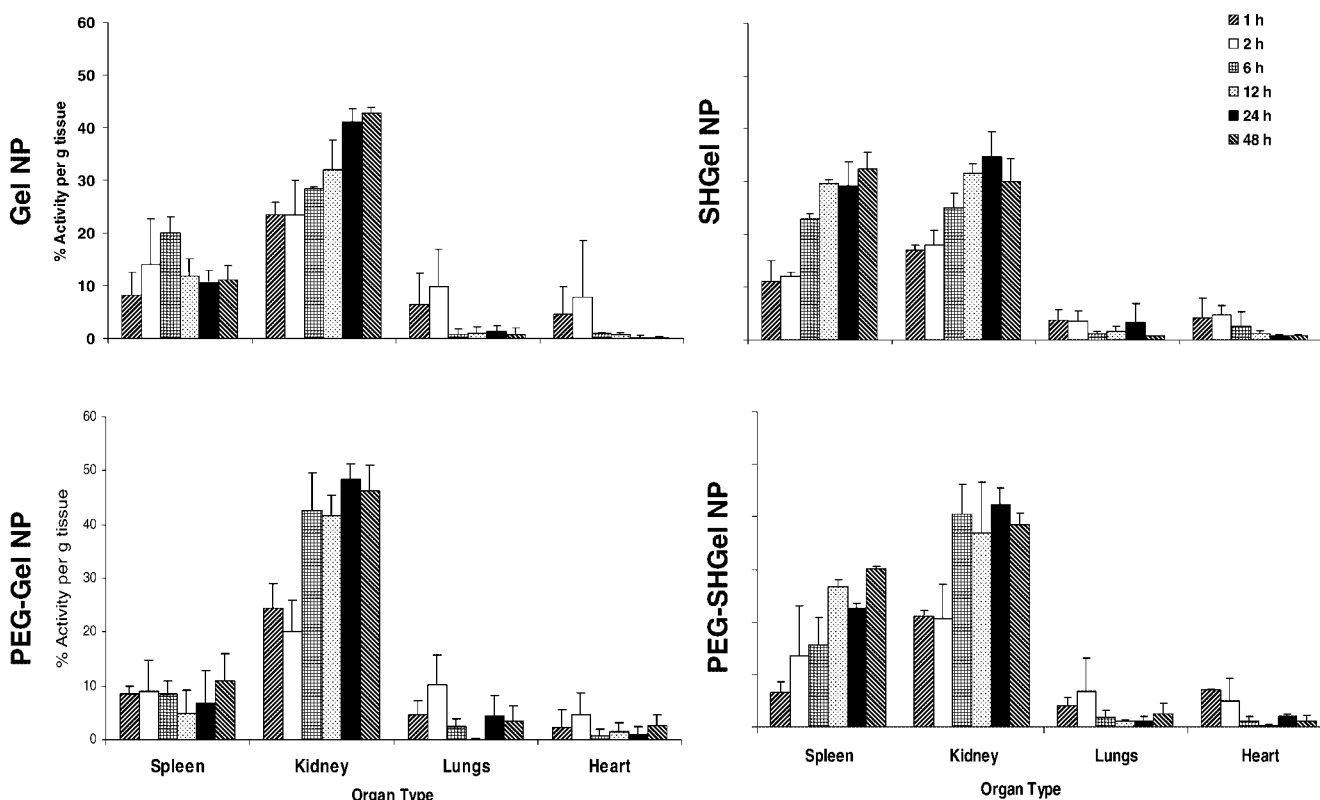


Figure 5. The change in nanoparticle concentrations as a function of time for up to 48 h in other highly perfused organs. ^{111}In -labeled gelatin, thiolated gelatin, poly(ethylene glycol)-modified gelatin, and poly(ethylene glycol)-modified thiolated gelatin nanoparticles were administered intravenously to female *Nu/Nu* mice bearing MDA-MB-435 human breast adenocarcinoma xenograft.

20% of the activity recovered per gram of tissue was found to be eliminated through spleen in the case of gelatin (Gel) nanoparticles, as compared to 32% in case of thiolated gelatin (SHGel) nanoparticles. Upon modification with PEG, the elimination of gelatin nanoparticles (PEG-Gel) was reduced by 10%, and that of thiolated gelatin (PEG-SHGel) by 5% of the activity recovered per gram of tissue. The results of the uptake of these nanoparticles by spleen show that the PEG modification of the thiolated gelatin nanoparticles had reduced their uptake by liver to a greater extent than in case of spleen, which is also mediated through the mechanism of RES. However, accumulation of the nanoparticles in lungs and heart was found to be negligible for all four tested formulations.

CONCLUSIONS

The thiolated gelatin nanoparticles have been successfully modified with the hydrophilic polymer PEG and the nanoparticles had a mean size of 290–350 nm. The disposition of radiolabeled PEG-modified nanoparticles confirmed their long circulation potential, when compared to their unmodified counterparts. The results of this study also show that the thiolated gelatin nanoparticles have sensitivity towards highly reducing environment, resulting in tumor-selective biodistribution. The PEG-modified thiolated gelatin nanoparticles with longer circulation times and enhanced tumor extravasation can, therefore, be used to target drugs and genes passively to solid tumors *in vivo*.

ACKNOWLEDGMENTS

This study was supported by a grant R01-CA095522 from the National Cancer Institute of the National Institutes of Health (NIH). The ESCA studies were performed at National ESCA and Surface Analysis Center for Biomedical Problems (NESAC/BIO), University of Washington, Seattle, WA. NESAC/BIO is supported by the NIH grant EB-002027.

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