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A short circulating peptide nanofiber as a carrier for tumoral delivery

Anil Wagh, PhD, Jagdish Singh, PhD, Steven Qian, PhD, Benedict Law, PhD*

Department of Pharmaceutical Sciences, North Dakota State University, Fargo, ND, USA

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Abstract

The cellular interactions and *in vivo* distribution of the nanomaterials are known to be strongly influenced by their physiochemical properties. Here, we investigated and compared the biocompatibility, pharmacokinetics, and biodistribution of previously reported peptide-based nanofiber (NFP), with commercially available nanomaterials. The NFP was a 2-dimensional (2D) structure with an extremely narrow width (4 nm) and a controllable length (50 to 400 nm). NFP was found to be non-toxic, hemocompatible, and with a minimum uptake by macrophages. *In vivo* studies further demonstrated that NFP could be delivered to the tumor site more effectively, and within a very shorter period of time, than spherical nanoparticles. Importantly, the undelivered NFP was rapidly eliminated by renal clearance and, thus, avoiding its accumulation in the spleen or liver. Overall, our data suggested a new paradigm in drug delivery *via* using a short circulating NFP, rather than a long circulating 3D nanoparticle, as a delivery cargo.

Key words: Nanofiber; Biocompatibility; Near-infrared imaging; Pharmacokinetics; Biodistribution

Many nanomaterials have been proposed as drug carriers.[1] Particularly in cancer, because of the leaky vasculature and poor lymphatic drainage, nanomaterials tend to accumulate at the tumor site *via* enhanced permeability and retention (EPR) effect.[2] For this reason, drug delivery using nanomaterials can offer an advantage of reducing adverse effects of chemotherapeutic agents. For example, paclitaxel, SN-38, doxorubicin, or cisplatin has been encapsulated into polymeric nanomaterials to reduce the administration dosage.[3–7] In fact, several nanoparticle formulations such as liposomal doxorubicin and albumin particles encapsulated with paclitaxel have been already approved by the food and drug administration (FDA) for the treatment of Kaposi's sarcoma, ovarian, and breast cancer.[8,9] However, due to the prolonged circulating time in the body, Doxil is known to suffer from new side effects, including hand-foot syndrome and mucositis. Furthermore, the pharmacokinetic and *in vivo* distribution of a nanomaterial can be affected by many physicochemical properties.[10]

Smaller nanoparticles (<3–5 nm) are often eliminated from the body by renal clearance and thus have relatively shorter plasma half-lives.[11] On the other hand, larger particles (>10–20 nm) are prompt to be captured by the reticuloendothelial system (RES),[12] and therefore, are more likely to be taken up by the liver and spleen.[13] Apart from size, the shape and charge of a nanomaterial can also play important roles in the pharmacokinetic property and biodistribution.[14,15] For example, after intravenous injection, the uptake of gold nanorods by liver was found to be less than its spherical counterpart of same size.[16] In another study, the negatively charged liposomes were shown to have a significantly shorter plasma half-life than the neutral ones.[15] Given that no universal rule can be applied to predict the *in vivo* behavior, the safety of the nanomaterials should be evaluated on a case by case basis. Besides, understanding the biocompatibility of a nanomaterial is essential to predict its future applications *in vivo*.

Peptide based nanomaterials have gained much interest because of design flexibility and structural diversity that enabled their

Abbreviations: EPR, enhanced permeability and retention; FDA, food and drug administration; RES, reticuloendothelial system; NFP, nanofiber; 2D, 2-dimensional; 3D, 3-dimensional; ASTM, American Society for Testing and Materials; SCID, severe combined immunodeficiency; QD, quantum dots; MWCT, multi-walled carbon nanotubes; PS, polystyrene nanoparticles; PLGA, pegylated poly(D,L-lactic-*co*-glycolic acid) nanoparticles; AU, gold nanoparticles; AUC, area under the curve; MRT, mean residence time; $t_{1/2}$, half-life; NIR, near-infrared.

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*Corresponding author: Department of Pharmaceutical Sciences, College of Pharmacy, Nursing and Allied Sciences, North Dakota State University, Fargo, ND, USA.

E-mail address: Shek.law@ndsu.edu (B. Law).

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Table 1
A table showing the physiochemical properties of different nanomaterials.

Nanomaterials	Material of construction	Dimension & shape	Size (nm)		Zeta Potential (mV)
			TEM	DLS	
NFP-50	Amino acid	2D Fiber	4 (w)×50 (l)	51±4 (l)	−10±2.0
NFP-100			4 (w)×100 (l)	85±5 (l)	−10±2.0
NFP-400			4 (w)×400 (l)	380±10 (l)	−10±2.0
PLGA-100	PLGA	3D Sphere	70 (d)	93±7 (d)	−15±3.0
AU-60	Gold	3D Sphere	60 (d)*	71±1 (d)	−34±2.5
AU-80			80 (d)*	90±1 (d)	−33±2.5
PS-50	Polystyrene	3D Sphere	50 (d)*	65±2 (d)	−33±2.5
PS-100			100 (d)*	110±3 (d)	−54±4.5
QD-6	Cadmium & selenium	3D Sphere	6 (d)*	27±2 (d)	−14±1.5
MWCT-2000	Carbon	3D Rod	10 (OD)×2000 (l)*	2580±150 (l)	−38±3.0

Abbreviations: Poly(D,L-lactic-*co*-glycolic acid)-polyethylene glycol (PLGA), transmission electron microscopy (TEM), dynamic light scattering (DLS), outside diameter (OD), width (w), length (l), diameter (d).
* Size determination by TEM was provided by the manufacturer.

diverse applications.[17–19] Self-assembling peptides have been employed as a novel platform for the local delivery of hydrophilic peptides, proteins, and hydrophobic anticancer agents.[20] For example, EAK16-11 is a class of self-assembling peptide, which can stabilize hydrophobic molecules such as pyrene and ellipticine and serve as an efficient slow delivery carrier for releasing the molecules in a controlled manner.[21] Similarly, our laboratory has previously developed a new type of peptide-based nanofiber (NFP) for imaging and drug delivery.[19,22] NFP was composed of multiple peptide constructs. Each peptide construct consisted of (a) a self-assembling peptide sequence (kldlkldlkldl) that has been used for tissue engineering[23] and (b) a hydrophilic polyethylene glycol (PEG) to prevent aggregation and possible uptake by the RES.[24] In an aqueous buffer, multiple peptides self-assembled together to form a nanofiber (NFP). Unlike the other nanofibers, NFP has a unique dimension (4 nm in width × 50–400 nm in length) and does not fuse to form the cylindrical nanotubes.[25] The presence of a hydrophilic mPEG chain also prevents the formation of a hydrogel network by inhibiting the possible interfibril interactions.[26]

Despite widespread use, there are very limited studies available for exploring the biocompatibility and tissue distribution of peptide-based nanomaterials. In the present study, we investigated the biocompatibility of previously designed NFP to extend the application to *in vivo* system. We also compared the hemocompatibility and biodistribution of NFP with various commercially available nanomaterials that were different in terms of composition, size, charge, surface area, and shape (Table 1). *In vivo* studies demonstrated that the optimized NFP was more effective as a tumoral delivery platform and, thus, have potentials as a delivery platform for cancer treatment.

Methods

MTS assay

MTS assay was performed as previously described.[19] Both mouse leukemic monocyte macrophage (RAW 264.7) and human embryonic kidney (HEK-293) cell lines were obtained from American Type Culture Collection (Manassas, VA, USA). The cells were cultured in Dulbecco's Modified Eagle Medium (DMEM) supplemented with 10% (v/v) fetal bovine serum (FBS), penicillin (50,000 units/L), and streptomycin (50 mg/L) and maintained in 5% CO_2 at 37 °C. The cells (5000/well) were seeded in a clear bottom 96-well plate (Corning Incorporated, Lowell, MA) for 12 h. The nanomaterials (0.4 and 2 mg/mL) in PBS buffer (50 μL) were mixed with complete DMEM medium (150 μL) and incubated with cells at 37 °C. Cells incubated with PBS buffer served as a negative control. After 24 h of incubation, fresh media containing the MTS reagent (Promega Corporation, Madison, WI) was added to each well and cells were further incubated for 1 h at 37 °C. The absorbance of the reduced formazan products (A) was directly measured at 490 nm using a spectrophotometer (Spectramax, Molecular Devices, Inc., Sunnyvale, CA, USA). All the absorbance values were corrected for the blank (media only). The interference of the respective nanomaterials at 490 nm was corrected by measuring the absorbance of same amount of the nanomaterial in the complete culture media. After all corrections, the percentage cell viability was determined by using the following equation:

$$\left(A_{sample}\right)/\left(A_{control}\right) \times 100\,\%.$$

Hemolysis assay

The hemolysis of the nanomaterials was tested according to new American Society for Testing and Materials (ASTM) standard E2525-08 as previously described.[27] Briefly, red blood cells (RBCs) were isolated from fresh citrated whole human blood by centrifugation (800 × *g*, 15 min), then washed and re-suspended in PBS buffer (3.5×10^8 cells/mL). The nanomaterials (0.2 and 1 mg/mL) in PBS buffer (100 μL) were added to the stock erythrocyte dispersion (100 μL) and then incubated for 3 h at 37 °C. Triton X-100 in deionized water (10% v/v) and PBS buffer were used as the positive and negative controls, respectively. The intact erythrocytes were then separated by centrifugation (800 × *g*, 15 min) and the absorbance (A) of the supernatant (100 μL) was measured at 540 nm to detect the released hemoglobin. The total hemoglobin concentration in the